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Identification of arachidonate metabolites in normal and malignant human lung

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Many tumours (see Bennett 1979) and normal tissues produce substantial amounts of prostaglandins. The prostaglandins in human normal lung and pulmonary carcinomas have not been formally identified previously; this paper describes our results with gas chromatography-mass spectrometry.

Materials and methods

Preparation of tissues. Tissues from five histologically confirmed carcinomas (two well differentiated and one poorly differentiated squamous carcinomas, and two undifferentiated large cell carcinomas), and five specimens of normal lung tissue removed with the tumours, were provided by a pathologist within 15 min of surgery. Each sample was cut into small pieces, washed in Krebs solution, and any carbon deposits > 3 mm were removed. Weighed amounts (1–3·5 g) were homogenized for 30 s at room temperature (20 °C) in Krebs solution (0·1 g ml^{-1}, Silverson homogenizer) to obtain new synthesis of cyclo-oxygenase and lipoxygenase products (collectively called eicosanoids) from released precursors (Bennett et al 1973).

Following chloroform extraction and evaporation (Unger et al 1971) part of each sample was bioassayed on rat gastric fundus against PGE_2. The remainder was dissolved in dichloromethane for purification on an LH20 column. Non-polar impurities were first removed by dichloromethane, and the eicosanoids eluted with methanol. These were evaporated to dryness, dissolved in 10 ml double-distilled water, acidified to pH 3·0 with 1 M hydrochloric acid, and percolated through Amberlite XAD-2 columns.

Unwanted substances were eluted with 15 ml distilled water followed by 5 ml n-heptane. The eicosanoids were eluted with 10 ml methanol, and the solvent removed by evaporation first at 40 °C under nitrogen, and then desiccated under vacuum. The residue was dissolved in 200 μl methanol—chloroform (1:1 v/v) and applied as a narrow band on a silica gel G thin-layer chromatography plate (200 × 100 × 0·2 mm Merck; ethyl acetate–acetone–glacial acetic acid, 95:5:1 by volume, Andersen 1969). Authentic prostanoid standards were applied to the zone next to each biological sample. The plates were developed to 15 cm from the origin and 1 cm zones were eluted twice with 5 ml methanol which was then evaporated. Zones corresponding to authentic arachidonic acid (AA) and 12-hydroxy-eicosatetraenoic acid (12-HETE) were pooled and the residues re-chromatographed as described above, using diethyl ether–light petroleum–acetic acid (50:50:1 by volume). This gave better separation of the zones corresponding to AA and 12-HETE which were eluted as described above. No search was made for leukotrienes (Samuelsson et al 1979).

The chloroform extraction method gives recoveries >70% with PGE, F and A compounds (Unger et al 1971). Recoveries on subsequent purification were about 60–80%.

Chemical derivatization. *O*-Methyloximes were formed by dissolving residues in 100 μl pyridine containing methyloxime hydrochloride (Aldrich) 5 mg ml^{-1}, and heating at 60–80 °C for 1 h. The pyridine was removed under vacuum for 30 min, and the *O*-methyloximes converted into the corresponding methylesters by dissolving them in 100 μl methanol and treating with 200 μl freshly redistilled diazomethane. After vortexing, the samples were evaporated under nitrogen at room

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Table 1. Bioassay and mass spectrometric determinations of acidic lipids extracted from normal lung (N) and tumour (T). Specimens 1–3 are squamous carcinomas (1 and 2, well differentiated, 3 poorly differentiated). Specimens 4 and 5 are undifferentiated large-cell carcinomas. Total and basal PG-lm represent the amount of biological activity (ng PGE_2 equivalents g^{-1} wet tissue) obtained respectively from homogenates in Krebs solution and acid ethanol. Response represents the speed of contraction of the assay tissue (S, slow as with PGE_2; F, faster response). Quantitative g.c.–m.s. determinations of prostaglandins D_2, E_2, $F_{2\alpha}$, 6-keto-$F_{1\alpha}$, are shown as ng g^{-1}. Qualitative g.c.-m.s. of 6,15-diketo-$F_{1\alpha}$, 6,15-diketo-13,14-dihydro-$F_{1\alpha}$, thromboxane B_2 and 15-keto-13,14-dihydro-thromboxane B_2 are shown in the bottom part of the Table. + full spectrum; NE not estimated; – below detection limit (about 80 ng g^{-1} wet weight tissue). No examination was made for leukotrienes.

	Specimens									
	1		2		3		4		5	
	N	T	N	T	N	T	N	T	N	T
Total PG-lm	110	130	12	256	44	18	14	1120	21	53
Basal PG-lm	38	27	7	65	16	9	10	950	10	33
Response	S	S	F	S	S	S	F	S	S	F
PGD_2	NE	NE	12	96	31	23	3	21	15	7
PGE_2	NE	NE	13	40	6	46	4	132	27	46
$PGF_{2\alpha}$	NE	NE	14	80	11	22	5	19	2	10
6-k-$PGF_{1\alpha}$	NE	NE	43	141	6	82	6	60	211	NE
6,15-k-$PGF_{1\alpha}$	NE	NE	+	+	+	+	+	+	+	+
6,15-k-diH-$PGF_{1\alpha}$	+	+	+	+	+	+	+	+	+	+
TxB_2	+	+	+	+	+	+	+	+	+	+
15-k-diH-TxB_2	+	+	+	+	+	+	+	+	+	+
AA	+	+	+	+	+	+	+	+	+	+
HETE	+	+	+	–	+	+	+	+	NE	NE

temperature and the procedure repeated. These esters were finally converted into trimethylsilyl ethers by heating them at 60 °C for 15 min in the presence of 25 µl *N,N*-bis (trimethylsilyl-tri-fluoracetamide) (BSTFA, Sigma).

Qualitative gas chromatography-mass spectrometry. G.c.-m.s. was performed with 10 µl aliquots of standards or samples injected into a Finnigan 9600 gas chromatograph equipped with a glass column (1·5 m × 2 mm) packed with 1% SE-30 on Supelcoport (phase separation). The chromatograph was interfaced via a glass jet separator with a Finnigan 3200 quadrupole mass spectrometer, and the system was operated using a Finnigan 6000 data system. The gas chromatograph temperature was between 175 ° and 220 °C, and helium (30 ml min^{-1}) was the carrier gas. Settings for the mass spectrometer were 25 eV electron energy, 10^{-7} amps V^{-1} pre-amplifier and 1700 V electron multiplier.

Several samples were analysed using a Riber 10-10C mass spectrometer with a Jirdel 31 gas chromatograph equipped with a 12·5 metre fused silica capillary column (Hewlett Packard SE30). Helium was used as the carrier gas at a flow rate of 2 ml min^{-1}, and the column temperature was 210–260 °C. The mass spectrometer was operated at 70 eV electron energy and an electron multiplier setting of 2200 V.

Quantitative measurements of PGD_2, PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ were made on four samples of tumour and associated normal tissue using the riber 10–10C m.s. for selective ion monitoring, and deuterated standards.

The prostanoid standards were obtained from The Upjohn Company. 12-HETE was prepared from blood platelets in co-operation with Dr P. Woollard; the presence of other HETE compounds has not been assessed.

Results

Bioassay. The amounts of prostaglandin-like material determined in extracts of tumour and normal tissue by rat fundus bioassay (Bennett et al 1973) were respectively equivalent in activity to 18–1120 and 12–110 ng PGE_2 g^{-1} wet weight. Some of this biological activity

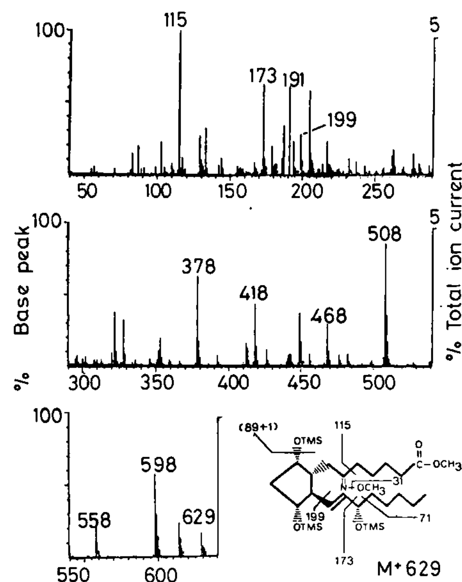

Fig. 1. Mass spectrum of 6-keto-$PGF_{1\alpha}$. The scale is magnified ×5 from 440 *m/z*, ×10 from 550 *m/z*, and ×40 from 610 *m/z*. The labelled peaks are M^+ minus the respective values as follows: 378, M^+ −((3 × 90) + 71); 418, (2 × 90) + 31; 468, (2 × 90) + 71; 508, 90 + 31; 558, 71; 598, 31.

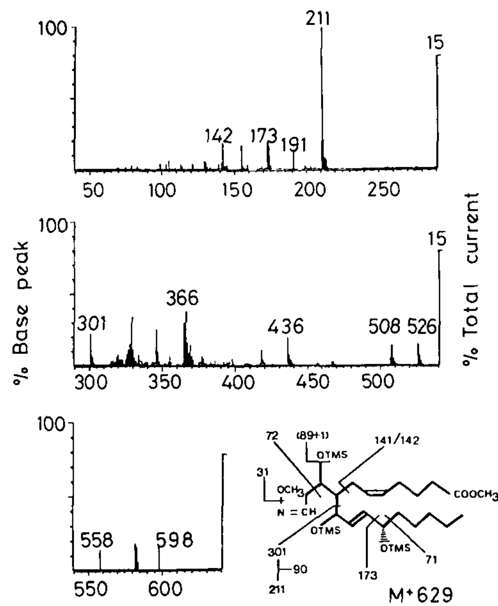

FIG. 2. Mass spectrum of TxB_2. The scale is magnified ×15 from 315 m/z, ×50 from 500 m/z, and ×500 from 550 m/z. The labelled peaks are the M^+ minus the respective values as follows: 366, M^+–(173 + 90); 436, 90 + 72 + 31; 508, 90 + 31; 526, 72 + 31; 558, 71; 598, 31.

extracted from one tumour, and from two other specimens of normal lung, was due to material which caused a faster response of the rat stomach bioassay tissue.

Mass spectrometry. The specimens of lung carcinomas and apparently normal lung tissue were obtained from five male patients aged 56–70 years. Results of g.c.-m.s. analysis for the tissue homogenates are shown in Table 1. Qualitative full mass spectra identical with authentic standards were obtained for the most eicosanoids (see Figs 1 and 2 for 6-keto-$PGF_{1\alpha}$ and TxB_2).

Only compounds formed from arachidonic acid were detected, and if metabolites of eicosatrienoic acid or eicosapentaenoic acid were present their recovered amounts were below the limits of detection (about 80 ng g^{-1} wet weight tissue for all prostanoids). All the extracts contained arachidonic acid, 6-keto-$PGF_{1\alpha}$, TxB_2 and, except for one tumour, 12-HETE. In addition, full spectra were obtained in each case for 6,15-diketo-13, 14-dihydro-$PGF_{1\alpha}$, 6,15-diketo-$PGF_{1\alpha}$ and 15-keto-13,14-dihydro-TxB_2. The presence of PGD_2, PGE_2 and $PGF_{2\alpha}$ were not often detected by full spectral scans, but they were found in all samples examined quantitatively by selective ion monitoring (Table 1).

Discussion

The bioassay results indicate that normal and malignant human lung tissues can produce substantial amounts of prostaglandin-like material. In addition to substances causing the slow contraction of rat gastric fundus obtained with most prostaglandins, some specimens yielded material which caused a faster contraction. The individual contributions to the biological activity of the extracts of this material and the various substances identified by g.c.–m.s. are not known. Many metabolites tested on the rat stomach are only weakly active, 6-keto-$PGF_{1\alpha}$, 6,15-diketo-$PGF_{1\alpha}$ and TxB_2 being at least 300 times less potent than PGE_2 (Bennett et al 1980).

Since all homogenates examined contained the lipoxygenase product 12-HETE, and all originally contained PGI_2 and TxA_2 as indicated by complete spectra of their degradation products 6-keto-$PGF_{1\alpha}$ and TxB_2, these eicosanoids seem to be the major substances formed by normal lung tissues, squamous carcinomas or large cell undifferentiated carcinomas when they are homogenized in physiological solution. Other arachidonate metabolites, particularly lipoxygenase products, may be present, but these could not be investigated since authentic standards were not available. It does not follow that the types and amounts of the substances detected reflect those normally produced by the tissue. Furthermore, the contribution of different cells to the amounts of metabolites is not known. For example, blood constituents and blood vessels are likely to contribute substantially to the amounts present in extracts. Nevertheless, in most cases the amounts of biologically active material and the separately measured quantities of PGD_2, PGE_2, $PGF_{1\alpha}$ and 6-keto-$PGF_{1\alpha}$ were greater in extracts of tumour than of normal tissue.

Normal and malignant lung tissue contains 15-hydroxy-dehydrogenase and Δ13-reductase, as shown by the presence of 6,15-diketo-$PGF_{2\alpha}$ and the 13,14-dihydro derivatives of 6,15-diketo-$PGF_{1\alpha}$ and 15-keto-TxB_2.

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