References

ALABASTER, VALERIE A. & BAKHLE, Y.S. (1970). Removal of 5-hydroxytryptamine in the pulmonary circulation of rat isolated lungs. *Br. J. Pharmac.*, **40**, 468–482.

SOUTHGATE, J. & COLLINS, G.G.S. (1969). The estimation of monoamine oxidase using ^{14}C-labelled substrates. *Biochem. Pharmac.*, **18**, 2285–2287.

UOTILA, P. & MARNIEMI, J. (1976). Variable effects of cigarette smoking on aryl hydrocarbon hydroxylase, epoxide hydrase and UDP-glucuronosyltransferase activities in rat lung, kidney and small intestinal mucosa. *Biochem. Pharmac.*, **25**, 2323–2328.

UOTILA, P., PELKONEN, O. & COHEN, G.M. (1977). The effects of cigarette smoke in the metabolism of ^{3}H-benzo[a]pyrene by rat lung microsomes. *Cancer Res.*, **37**, 2156–2161.

U.S. DEPARTMENT OF HEALTH, EDUCATION AND WELFARE (1975). The health consequences of smoking. Public Health Service, Washington, D.C.

WELCH, R.M., LOH, A. & CONNEY, A.H. (1971). Cigarette smoke; stimulatory effect on metabolism of 3,4-benzo-[a]pyrene by enzymes in rat lung. *Life Sci.*, **10**, 215–221.

Tumour growth and response to treatment: beneficial effect of the prostaglandin synthesis inhibitor flurbiprofen

A. BENNETT, J. HOUGHTON, D.J. LEAPER & I.F. STAMFORD

Department of Surgery, King's College Hospital Medical School, London SE5 8RX.

Prostaglandins (PG) are implicated in the growth and spread of some tumours. It is important to study inhibitors of PG synthesis for this reason, and because patients may take such drugs. Indomethacin reduces the growth of tumours induced in mice by Moloney sarcoma virus (Strausser & Humes, 1975). We report a similar effect with flurbiprofen, and show in addition a tendency to increased survival time and a significant enhancement of the tumour response to radiotherapy and chemotherapy.

WHT/Ht albino mice of either sex were injected sc into the right flank with 10^6 cells of the spontaneous non-immunogenic murine WHT-NC tumour. Flurbiprofen (5 mg/kg) was given orally once each day, where indicated, in 0.1 ml raspberry syrup (V. Eisen & D.I. Walker, personal communication). Tumours, metastases, and recurrences were removed, weighed, and homogenized in Krebs solution. PGs were extracted and bioassayed on the rat gastric fundus strip preparation against PGE_2 (see Bennett, Stamford & Unger, 1973). Unless stated otherwise, the results are means ± s.e. mean.

Tumour growth and mouse survival were studied in the following groups: (1) tumour inoculation only ($n = 10$); (2) tumour inoculation + flurbiprofen treatment throughout ($n = 10$); (3) tumour excision at 3 weeks ($n = 30$); (4) tumour excision at 3 weeks, then flurbiprofen treatment ($n = 10$); (5) tumour excision at 3 weeks, flurbiprofen treatment throughout ($n = 19$). Primary tumour widths (W) and lengths (L) (groups 1 and 2) were measured weekly, and volumes calculated as $\pi.W^2.L/6$. Flurbiprofen treatment reduced the tumour weights, and tended to prolong survival in mice whose primary tumour was removed (Table 1).

The response of tumours to treatment was studied in groups of mice given 1, 2, or 3 of the following: chemotherapy (melphalan 0.15 mg/kg, days 30–32 and 37–39 after tumour inoculation, and methotrexate

Table 1 The effect of flurbiprofen on tumour weight and mouse survival time

Group	*Primary tumour weight (g) (at 3 weeks groups 3–5, or at death groups 1 and 2)*	*Primary tumour PGs (ng PGE_2 equivalents/g)*	*Survival (days) medians and semi-quartile ranges*
1	3.66 ± 0.15	85 ± 26	73(70–77)
	($P < 0.05$)	($0.1 > P > 0.05$)	($P = 0.327$)
2	1.89 ± 0.21	32 ± 5	76(67–82)
3	0.25 ± 0.03	469 ± 53	70(68–84)*
	($P < 0.005$)	($P < 0.001$)	
4			77(62–86)
5	0.14 ± 0.03	36 ± 13	83(70–91)*†

* $P = 0.068$, Mann–Whitney U-test.
† Two still alive at 127 days.

0.2 mg/kg, days 30 and 37), local radiotherapy (500 rads, days 30 and 37), and flurbiprofen, day 25 onwards. The tumours were excized after 6 weeks.

The extracts of tumours from mice given flurbiprofen contained 60 ± 16 to 74 ± 30 ng PGE_2 equivalents/g wet tumour, compared with 125 ± 19 ng/g in controls (all $P < 0.02$). Tumour weights were similar to controls (0.99 ± 0.19 g) except in mice receiving flurbiprofen with radiotherapy (0.45 ± 0.05 g) or radiotherapy + chemotherapy (0.29 ± 0.05 g) ($P < 0.02$ and < 0.01 respectively).

We conclude that flurbiprofen reduced the growth of the primary tumours and their ability to synthesize PGs, tended to increase survival time following removal of the primary tumour, and increased the effect of radiotherapy and chemotherapy.

We thank the C.R.C. and M.R.C. for support.

References

BENNETT, A., STAMFORD, I.F. & UNGER, W.G. (1973). Prostaglandin E_2 and gastric acid secretion in man. *J. Physiol. (Lond.)*, **229**, 349–360.

STRAUSSER, H.L. & HUMES, J.L. (1975). Prostaglandin synthesis inhibition: effect on bone changes and sarcoma tumour induction in BALB/c mice. *Int. J. Cancer*, **15**, 724–730.

C.21

The effects of prostaglandin D_2 on the circular muscle of guinea-pig isolated ileum and colon

A. BENNETT & G.J. SANGER

Department of Surgery, King's College Hospital Medical School, London SE5 8RX.

Prostaglandin (PG)D can be formed by gastrointestinal tissue (Nugteren & Hazelhof, 1973). PGD_2 contracts the gut longitudinal muscle of various species (e.g. Hamberg, Hedqvist, Strandberg, Svensson & Samuelsson, 1975). Apart from rabbit stomach (Moncada, Mugridge & Whittle, 1977) the effects of PGD_2 on gastrointestinal circular muscle have not been studied. We report experiments on circular muscle of guinea-pig ileum and colon. Spiral strips approximately 3 mm wide and 30 mm long were cut from the distal ileum and distal colon of male guinea-pigs weighing approximately 400 g. Each strip was suspended under a load of 1 g in Krebs solution at 37°C bubbled with 5% CO_2 in O_2. Isotonic responses were measured using transducers and pen recorders.

The circular muscle strips of guinea-pig ileum had no tone, and since PGD_2 did not cause a contraction we looked for an inhibitory effect on submaximal responses elicited with 1.5 mg/ml KCl.

PGD_2, PGE_2 or $PGF_{2\alpha}$ (1 μg/ml) given 1 min before the KCl reduced the contraction to KCl by 21 ± 6, 90 ± 3 and 5 ± 2% (mean ± s.e. mean; $n = 8$, 7 and 7 respectively); PGD_2 2 μg/ml reduced the response to KCl by 37 ± 4% ($n = 7$). The findings with PGE_2 and $PGF_{2\alpha}$ confirm those of Bennett, Eley & Scholes (1968) and Bennett, Eley & Stockley (1975).

In colonic circular muscle PGD_2, by contrast, caused contraction, 70 ± 10 ng/ml being required for a threshold contraction ($n = 9$). As found by Fleshler & Bennett (1969), $PGF_{2\alpha}$ contracted this tissue (threshold concentration; 8 ± 2 ng/ml, $n = 9$) and PGE_2 caused relaxation. We confirmed that the PG antagonist SC-19220 blocks the contractions to $PGF_{2\alpha}$ but not the relaxations to PGE_2 (Bennett & Posner, 1971). SC-19220 (80–130 ng/ml) also prevented the contraction to PGD_2 (1 μg/ml), but it greatly reduced muscle tone and hampered detection of relaxation. However, PGD_2 (1 μg/ml) now reduced submaximal contractions to acetylcholine by 29 ± 10% ($n = 4$) whereas in the presence of $PGF_{2\alpha}$ (1 μg/ml) the acetylcholine-induced contractions were virtually unchanged (101 ± 3% of controls, $n = 4$).

Thus, in colonic circular muscle, PGD_2 exerted a predominant excitatory '$PGF_{2\alpha}$-like' activity which overshadowed the inhibitory 'PGE_2-like' response. However, in the ileum circular muscle only a 'PGE_2-like' response occurred with PGD_2, and this tissue is virtually unresponsive to $PGF_{2\alpha}$.

We suggest that there are regional differences in the distribution of receptors activated by PGD, E and F_α compounds, that the 9-hydroxyl group is important for activation of receptors stimulated by $PGF_{2\alpha}$, and that a 9- or 11-oxo group is important for activation of receptors stimulated by PGE_2.

References

BENNETT, A., ELEY, K.G. & SCHOLES, G.B. (1968). Effects of prostaglandins E_1 and E_2 on human, guinea-pig and rat isolated small intestine. *Br. J. Pharmac.*, **34**, 630–638.

BENNETT, A., ELEY, K.G. & STOCKLEY, H.L. (1975). The effects of prostaglandins on guinea-pig isolated intestine and their possible contribution to muscle activity and tone. *Br. J. Pharmac.*, **54**, 197–204.