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Acetylsalicylic acid (ASA) protects the prostaglandin-cAMP-system of human hypernephroma cells against irradiation-induced alterations

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Summary There is abundant evidence that inhibitors of prostaglandin (PG) biosynthesis might increase the radioresponse of certain tumour cells. This study investigated specific PG binding sites, eicosanoid production as well as intracellular cAMP levels in cultured human hypernephroma cells derived from 11 patients upon nephrectomy. Scatchard analyses of the binding data revealed specific PGE_1-, PGE_2- as well as PGI_2-binding sites (PGE_1: $B_{max} = 755 \pm 206$ fmol mg^{-1} protein, $K_d = 3.7 \pm 2.7$nM PGE_2: $B_{max} = 494 \pm 221$ fmol mg^{-1} protein, $K_d = 4.2 \pm 2.5$nM; PGI_2: $B_{max} = 693 \pm 164$ fmol mg^{-1} protein, $K_d = 6.0 \pm 4.5$nM). Significant ($P < 0.01$) increase in PG binding sites expressed on human hypernephroma cells (PGE_1: $B_{max} = 1084 \pm 303$ fmol mg^{-1} protein, $K_d = 2.8 \pm 1.3$nM; PGE_2: $B_{max} = 663 \pm 309$ fmol mg^{-1} protein, $K_d = 2.2 \pm 1.5$nM; PGI_2: $B_{max} = 1021 \pm 391$ fmol/protein, $K_d = 4.2 \pm 3.6$nM) and inhibition of PG biosynthesis (TXB_2: -82.5%, PGE_2: -87.5%. PGD_2: -80.6%, PGF_{2a}: -81.3%) were found after acetylsalicylic acid (ASA)-treatment (0.5 mg 10^{-6} cells for 24 h). Following irradiation (^{60}Co, 1.0 Gy/min^{-1} over 10 min), PG binding sites (PGE_1: $B_{max} = 266 \pm 153$ fmol mg^{-1} protein, $K_d = 5.0 \pm 5.0$nM; PGE_2: $B_{max} = 148 \pm 66$ fmol mg^{-1} protein, $K_d = 4.7 \pm 3.6$nM; PGI_2: $B_{max} = 325 \pm 194$ fmol mg^{-1} protein, $K_d = 6.8 \pm 7.1$nM) were significantly ($P < 0.01$) diminished. However, irradiation had no significant effect on PG bindig sites in ASA-pretreated cells (PGE_1: $B_{max} = 699 \pm 240$ fmol mg^{-1} protein, $K_d = 3.5 \pm 1.8$nM; iloprost: $B_{max} = 766 \pm 452$ fmol mg^{-1} protein, $K_d = 3.2 \pm 2.2$nM). Although there was no significant difference in the basal values for cAMP between control and ASA-treated group cells, the PG-induced cAMP-production was less pronounced in the control group.

Taken together, the findings suggest that ASA may modify the radioresponse of cultured human hypernephroma cells by preventing the decrease of PG binding sites induced by irradiation.

Prostaglandins (PGs) are considered to play an important role in the regulation of tumour cell growth and metastases formation (Honn *et al.*, 1981). In recent years several studies have shown that inhibitors of PG biosynthesis, including indomethacin, may improve the therapeutic effect of chemo- (Powles *et al.*, 1978), immuno- (Chun & Hoffman, 1987) and/or radiotherapy (Furuta *et al.*, 1988*a*) regimens of some tumours. However, whereas indomethacin remarkably increased tumour cell radioresponse, it had only minimal effect on the radioresponse of normal tissues such as hair follicles, jejunum or hematopoietic tissue (Furuta *et al.*, 1988*a*). Furthermore, the response to indomethacin treatment was dependent on the ability of the tumour to produce PGs, mainly PGE_2 and PGI_2 (Furuta *et al.*, 1988*b*). Apart from the fact that the mechanisms by which indomethacin potentiates tumour radioresponse are still unclear to date, it appears that an increased tumour response might be achieved by lowering PGs in the tumour. This, however, would imply that PGs are not radioprotective agents as has been suggested by Hanson & Ainsworth (1985) or Walden *et al.* (1987). Milas and coworkers (1990) have also found that potentiation of the tumour radioresponse induced by indomethacin is more significant when it is given after rather than before irradiation. Beside recent reports on the dependence of indomethacin-augmented radioresponse on immunocompetence of the tumour host (Milas *et al.*, 1990), another possibility for PG action on tumour cells would be radiosensitisation by PGs. Moreover, one has also to bear in mind that the PG production may be remarkably different among various tumours (Malachi *et al.*, 1981; Ziboh *et al.*, 1981).

PGs exert their effects after interaction with specific cell surface receptors (Virgolini *et al.*, 1992). We have demonstrated in human thyroid cancer (Virgolini *et al.*, 1988) and hepatomas (Virgolini *et al.*, 1989) that the number of PG receptors significantly ($P < 0.001$) correlates with the cellular differentiation of the tumour. In these studies we found that high differentiated cancers seems to possess a higher PG receptor density than do anaplastic or less differentiated cancers. Furthermore, patients with a higher PG receptor density might have a better clinical prognosis. We have now used a similar receptor assay and have characterised the PG receptor on cultured human hypernephroma cells. These cells were shown to produce an increased amount of PGs of the E-series as compared with normal tissue (Cummings & Robertson, 1977). Furthermore, hypernephroma cells are relatively radiosensitive to high-dose radiation therapy (Halperin & Harisindis, 1983; Lang & deKernion, 1981). In a further step we wondered to what extent the PG system (PG receptors, cAMP-formation, PG production) would be influenced by irradiation, and investigated the effects of acetylsalicylic acid (ASA) on this system.

Materials and methods

Hypernephroma cell culture preparation

Tissue specimens (approximately 1 cm^3) were obtained intraoperatively from 11 patients aged 61 ± 12 years (nine males and two females) undergoing nephrectomy for renal cell carcinomas. Five of the patients had metastatic cancer, four were free of metastases. All patients had given written and informed consent to the study. From each patient a cell line was cultured. Renal cell carcinoma tissue specimen were made into a cell suspension by an enzymatic procedure as follows: suspended cells were incubated on a Petri dish in a humidified atmosphere of 2.5% CO_2, 97.5% O_2 at 37°C. The growth medium was changed three times a week. When the cells were confluent, a cell suspension was prepared by incubation with trypsin-EDTA-solution (0.05% trypsin, 0.02% EDTA) in phosphate buffered saline (PBS). Cells were routinely maintained in medium consisting of nutrient mixture F12 (Ham) supplemented with 12.5% fetal bovine serum, 0.24 mg collagenase, 0.01 mg DNA-se, 100 I.U. penicillin and 100 µg streptomycin ml^{-1}. The cells were plated into 6 cm plastic Petri dishes and stored in the incubator for 4-18 days for attachment and growth. When the Petri dishes were densely covered with cells, a single-cell suspension was prepared. One part of the cell suspension was plated for further propagation, another part was used for

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testing the multiplication of cells with different concentrations of Cytochalasin B (CB) and one part was plated into plastic tissue-culture chambers (Lab-Tek), fixed after 24–72 h and checked for content of cytokeratin as an indicator of malignancy (Diereck *et al.*, 1991). If there were only cytokeratin-positive cells, the cell culture was used for combined testing in the second passage. Cultures which were contaminated with cytokeratin-negative cells were further propagated and checked again when the cells became confluent. If there were still cytokeratin-negative cells after the fourth passage, the cell line was excluded from the test.

Cells from each cell line (one from each patient) were divided into four groups:

(1) Control group;
(2) ASA-treated group: cells were cultured in the presence of ASA (Bayer, Leverkusen, Germany; concentration 0.5 mg^{-6} cells ml^{-1} medium for 24 h);
(3) Control group irradiated: irradiation was performed with gamma rays from a ^{60}Co unit (Gammatron, Siemens) with a source-surface distance of 75 cm and a dose rate of 1.0 Gy min^{-1} over 10 min. Dose measurements (calibrations) were carried out with a standard dosimeter (Farmer 0.6 cm, Nucl. Enterprise).
(4) ASA-treated group irradiated: the same irradiation scheme as in group 3.

Cellular viability was assessed by phase-contrast microscopy and Trypan Blue exclusion criteria (Wandl *et al.*, 1989).

Binding studies

PG receptor binding studies were carried out according to the methodology described previously (Virgolini *et al.*, 1992). All assays reported here were performed approximately 2 h after irradiation. Briefly, the cells ($4-6 \times 10^6$ cells in each group) were washed twice in assay buffer containing 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1 M NaCl and centrifuged at 500*g* for 10 min at 4°C (Beckman J-6B Centrifuge, München, Germany). The pellet was suspended in 4°C assay buffer at a protein concentration of approximately 300 μg membrane protein ml^{-1} (200–420 μg ml^{-1}) as determined by the assay kit provided by Bio-Rad Laboratories (Coomassie Brilliant Blue G-250, Richmond, CA). In preliminary experiments the time course of binding as well as the dependency of binding on temperature was studied. Based on the results of these experiments all further incubations were performed at 4°C for 50 min to ensure equilibrium.

In saturation experiments hypernephroma cells (300 μg protein ml^{-1}) were incubated either with increasing concentrations of ^{3}H-iloprost (a chemically stable PGI_2-analog (Skuballa & Vorbrüggen, 1983); specific activity 14.7 Ci $mmol^{-1}$; radiochemical purity 98.7%; 0.5–100 nM), ^{3}H-PGE_1 (specific activity 42 Ci $mmol^{-1}$; radiochemical purity 97.5%; 0.5–100 nM) or ^{3}H-PGE_2 (specific activity 143 Ci $mmol^{-1}$; radiochemical purity 97.5%; 0.5–98 nM), respectively, in order to determine total binding (TB). In separate incubation series these increasing concentrations of ^{3}H-iloprost, ^{3}H-PGE_1 or ^{3}H-PGE_2 were incubated in the presence of the same unlabelled agonist to determine nonspecific binding (NSB). Specific binding (SB) was expressed as the difference of total and nonspecific binding. In displacement studies cells (300 μg ml^{-1} protein) were incubated with 15 nM of ^{3}H-PGE_1, ^{3}H-PGE_2 or ^{3}H-iloprost in the absence (TB) and presence of increasing concentrations (10^{-9}–10^{-4} M) of unlabelled agonist (NSB).

After incubation for 50 min at 4°C the reaction was diluted rapidly with 4 ml of 4°C assay buffer and filtered through a Whatman GF/B filter (Whatman Inc., Clifton, NY) under reduced pressure (−60 kPa). The filters were then dried, transferred into scintillation vials (Packard, Downers Grove, IL) and taken up into 10 ml scintillation fluid (Pico-Flour TM30, Packard). The radioactivity in the samples was counted for 5 min in a liquid scintillation counter (LKB Wallace, 1215 Rackbeta, Finland).

^{3}H-iloprost, ^{3}H-PGE_1, ^{3}H-PGE_2 and iloprost were obtained from Amersham International, Buckinghamshire, UK. Unlabelled PGE_1 and PGE_2 were purchased from The Upjohn Company, Kalamazoo, MI.

The inter-assay and intra-assay variability amounted to 6.2 ± 2.7% and 3.9 ± 2.3%, respectively.

Measurement of cAMP formation

Four to 6×10^6 tumour cells in each group were washed twice in 50 mM Tris-HCl buffer (pH 7.5) and resuspended in 50 mM Tris-HCl buffer (pH 7.5, 4°C) containing theophylline (BYK, Gulden Konstanz, Germany, 120 mg l^{-1}) to block the phosphodiesterase and ASA (50 mg l^{-1}) to prevent endogenous PG synthesis.

Cells were incubated in a 37°C shaking water bath for 30 min with either PGE_1, PGE_2 or iloprost in a concentration range from 10^{-4} M to 10^{-9} M. After 30 min the cells were homogenised by ultrasound (Sonicator, W-220F, Plainview, NY) and ultraturrax (TP18/10, Janke & Kunkel GmbH, Staufen, Germany) for 10 s. The incubation was stopped by centrifugation at 5000 *g* for 10 min at 4°C. The cAMP concentration in the supernatant was determined by RIA according to the manufacturer's description (Amersham). Briefly, the samples were mixed with ^{125}I-cAMP and rabbit anti-cAMP serum and incubated for 3 h at 4°C. The antibody-bound cAMP was then extracted with a donkey anti-rabbit antibody, which was coated onto magnetisable polymer particles. These were mixed and left to react with the antibodies for 10 min at 25°C. The antibody-bound fraction was obtained with a magnetic separator (Amerlex-M Separator, Amersham) and the activity was determined by a gamma counter (Riastar, Packard). The intra-assay variability was 3.5 ± 1.3% and the inter-assay 5.6 ± 2.3%.

Radiothinlayer chromatography

The cells ($4-6 \times 10^6$ in each group) were resuspended in 50 mM Tris-HCl buffer (pH 7.5) and incubated with 0.25 μCi ^{14}C-arachidonic acid (AA) (New England Nuclear, Boston, MA; specific activity 54.5 mCi $mmol^{-1}$; radiochemical purity 96.3%) for 30 min in a shaking water bath (37°C). The reaction was stopped with 1 M HCl. After centrifugation at 5000 *g* (10 min, 4°C) the supernatant was extracted with ethylacetate (Merck, Germany), dried under nitrogen, redissolved in absolute ethanol and stored at −20°C for not

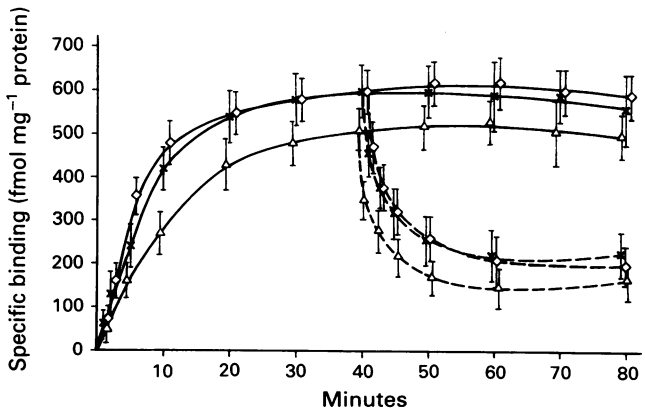

Figure 1 Time course of specific ^{3}H-PG binding to human hypernephroma cells. Association: ^{3}H-iloprost (*), ^{3}H-PGE_1 (◇) (each 84 nM) or ^{3}H-PGE_2 (△) (45 nM) was incubated in absence (total binding) and presence (nonspecific binding) of unlabelled agonist (100 μM). Specific binding (shown) was defined as the difference of total and nonspecific binding. Dissociation: at equilibrium an excess of the same unlabelled agonist was added. ^{3}H-PGs were displacable within 10 min. Each point represents the mean ± s.d. of four independent experiments with hypernephroma cells from different cell lines.

longer than 14 days. The samples were then automatically (Lamag-Linomat, Hiltons-Syringe, Hamilton, Bonaduz, Switzerland) placed onto a silica gel plate (Merck) together with the standards (PGE_2, $PGF_{2\alpha}$, 12-HETE, TXB_2, PGD_2, New England Nuclear). The plate was put into a glass chamber with the lower edge in organic phase which consisted of chloroform, acetic acid, H_2O and methanol (90:1:0.7:8) until the organic phase had reached the upper edge. Then the plate was removed and analysed by an automatic TLC-analyser (LB283, Berthold, Wilbad, Germany) coupled with an oscilloscope (Time ADL, Berthold).

Analysis

Binding data were analysed according to Scatchard (1949) using a computer programme which searched systematically for the highest level of correlation under the model of two straight lines in the given interval and tested against the alternative of single straight line approximation. Values are presented as means ($\bar{x}$) ± standard deviations (s.d.).

Statistical analysis was done by standard statistical tests including Student's t test and ANOVA at a confidence level of 95%.

Results

Binding of ^{3}H-iloprost, ^{3}H-PGE_1 and ^{3}H-PGE_2 to cultured human hypernephroma cells

In initial experiments the time course of ^{3}H-iloprost, ^{3}H-PGE_1 and ^{3}H-PGE_2-binding to human hypernephroma cells was investigated showing a constant increase of ^{3}H-ligand specifically bound during the first min of incubation (Figure 1). Equilibrium was reached within 20 min in ^{3}H-iloprost and ^{3}H-PGE_1 binding experiments and within 30 min in ^{3}H-PGE_2 binding experiments, and remained stable for at least 80 min. No significant numbers of cells were lost over the time scale of the experiments.

Specific ^{3}H-iloprost, ^{3}H-PGE_1 and ^{3}H-PGE_2 binding was only slightly dependent on temperature and was insignificantly lower at 37°C and at 22°C than at 4°C.

^{3}H-iloprost, ^{3}H-PGE_1 and ^{3}H-PGE_2 binding to human hypernephroma cells could be displaced by unlabelled iloprost, PGE_1 and PGE_2, respectively. In ^{3}H-iloprost binding studies the concentrations of unlabelled iloprost, PGE_1, PGE_2 for causing half maximal inhibition (IC_{50}) were $81 \pm 25 \times 10^{-9}$ M, $12 \pm 6 \times 10^{-8}$ M and $9 \pm 3 \times 10^{-6}$ M, respectively. The IC_{50} values were $40 \pm 10 \times 10^{-9}$ M for PGE_1, $86 \pm 20 \times 10^{-9}$ M for iloprost, and $85 \pm 26 \times 10^{-6}$ M for PGE_2 in ^{3}H-PGE_1 binding experiments, and amounted to $42 \pm 9 \times 10^{-9}$ M for PGE_2, $4 \pm 2 \times 10^{-6}$ M for PGE_1 and $30 \pm 7 \times 10^{-6}$ M for iloprost in ^{3}H-PGE_2 binding studies (Figure 2a–c).

Saturation of ^{3}H-PG binding to human hypernephroma cells was studied by incubating increasing concentrations of ligand in the absence and presence of an excess of the same unlabelled agonist. Binding was saturable and indicated a single class of high affinity binding sites for all three ligands within the ligand range studied (Figure 3a–b). After preincubation of the cells with ASA (group 2) a significantly ($P<0.01$) increased capacity to bind the PGs was found. The respective binding data are given in Tables I–III showing that ASA treatment increased ^{3}H-iloprost receptors from 693 ± 164 to 1021 ± 391 fmol mg^{-1} protein ($P<0.05$), ^{3}H-PGE_1-receptors from 755 ± 206 to 1084 ± 303 fmol mg^{-1} protein ($P<0.01$) and ^{3}H-PGE_2-receptors from 494 ± 221 to 663 ± 309 fmol mg^{-1} protein ($P<0.05$). Increase in the binding capacity was accompanied by a significant ($P<0.05$) decrease in the dissociation constant K_d.

In order to investigate the effect of irradiation on hypernephroma cells, irradiation with ^{60}CO at a dose rate of 1 Gy min^{-1} was performed. All binding experiments were carried out at approximately 2 h after irradiation. At this time cellular viability was unchanged. Irradiation caused a

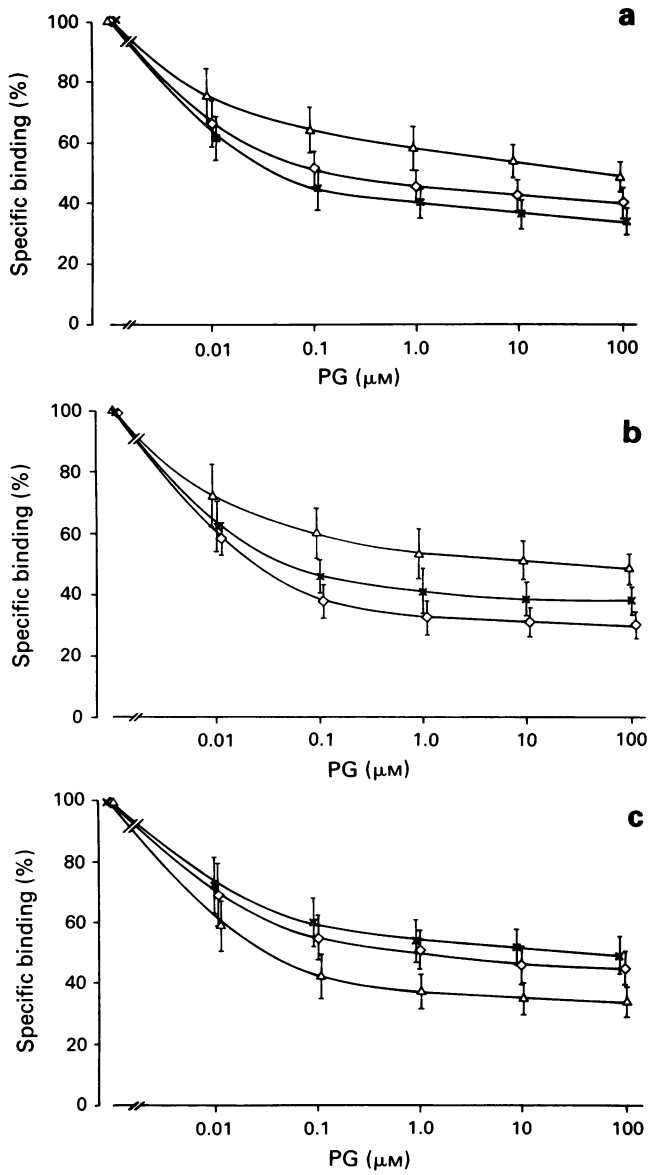

Figure 2 **a–c** Ability of unlabelled PG to compete with ^{3}H-PG for binding to human hypernephroma cells. Each assay tube contained 15 nM ^{3}H-PG (^{3}H-iloprost **a**; ^{3}H-PGE_1 **b**; ^{3}H-PGE_2 **c**) and the indicated concentrations of corresponding unlabelled agonist (iloprost (*), PGE_1 (◇) and PGE_2 (△)) Each point represents the mean ± s.d. of three independent experiments from different cell lines.

significant decrease in ^{3}H-PG-receptors: ^{3}H-iloprost receptor decreased to 325 ± 194 fmol mg^{-1} protein ($P<0.001$), ^{3}H-PGE_1-receptors to 266 ± 153 fmol mg^{-1} protein ($P<0.001$), and ^{3}H-PGE-receptors to 148 ± 66 fmol mg^{-1} protein ($P<0.01$). After pre-treatment with ASA the decrease induced by irradiation was much less pronounced ($P>0.05$) (^{3}H-iloprost receptors: 766 ± 452 fmol mg^{-1} protein, ^{3}H-PGE_1-receptors: 699 ± 240 fmol mg^{-1} protein, ^{3}H-PGE_2-receptors: 287 ± 114 fmol mg^{-1} protein).

Effect of PGs on cAMP-formation

There was no significant difference in basal values between control (30.1 ± 10.5 pmol mg^{-1}) and ASA-treated group (28.0 ± 11.5 pmol mg^{-1} protein). However, the basal values of control group irradiated (17.6 ± 8.9 pmol mg^{-1} protein) and of ASA-treated group irradiated (15.8 ± 6.1 pmol mg^{-1} protein) were significantly ($P<0.001$) lower as compared to the control group.

Iloprost, PGE_1 and PGE_2 significantly ($P<0.01$) stimulated cAMP-production in all four groups dose-dependently. However, the half maximal effective doses (ED_{50})

were significantly different between the four groups (Figure 4a–c). The corresponding ED_{50} values for iloprost were $12 \pm 8 \times 10^{-5}$ M for the control group, $35 \pm 13 \times 10^{-7}$ M for the ASA-treated group, $80 \pm 21 \times 10^{-7}$ M for the control group irradiated, and $43 \pm 11 \times 10^{-7}$ M for the ASA-treated group irradiated. The ED_{50} for PGE_1 were $25 \pm 8 \times 10^{-5}$ M for the control group, $30 \pm 10 \times 10^{-7}$ M for the ASA-treated group, $11 \pm 6 \times 10^{-7}$ M for the control group irradiated, and $88 \pm 15 \times 10^{-7}$ M for the ASA-treated group irradiated. The corresponding ED_{50} values for PGE_2 were $11 \pm 4 \times 10^{-5}$ M for the control group, $61 \pm 22 \times 10^{-8}$ M for the ASA-treated group, $86 \pm 32 \times 10^{-7}$ M for the control group irradiated, and $33 \pm 12 \times 10^{-8}$ M for the ASA-treated group irradiated.

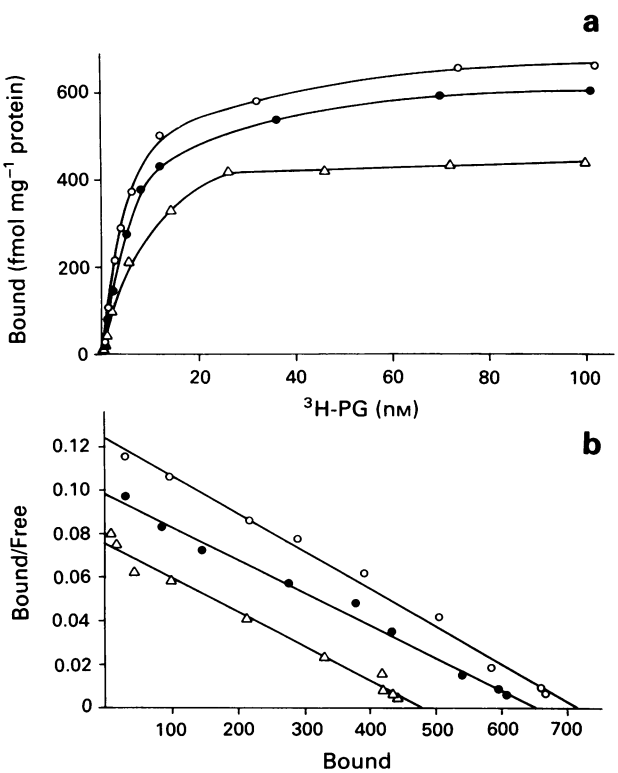

Figure 3 **a–b** Saturation experiments of ^{3}H-PG binding with human hypernephroma cells. **a**, Saturation curve of specific ^{3}H-PG binding. **b**, Corresponding Scatchard analysis. Each point represents the mean of six independent experiments with hypernephroma cells from different cell lines. (●) ^{3}H-iloprost binding; (○) ^{3}H-PGE_1-binding; ^{3}H-PGE_2-binding (△).

Conversion of exogenously added ^{14}C-AA to its metabolites

Hypernephroma cells converted exogeneous precursor AA to a various number of compounds (HETE, PGD_2, thromboxane B_2 (TXB_2), PGE_2, $PGF_{2\alpha}$) (Table IV). The main metabolites of AA conversion of cultured hypernephroma cells were PGE_2 and TXB_2 in both the control and the irradiated groups. After ASA treatment, PG production was significantly decreased – the main metabolite of AA conversion was HETE.

Discussion

Previously, we have identified and described PG binding sites on tumour cells (Virgolini *et al.*, 1988, 1989) and have demonstrated that cellular differentiation could be closely associated with receptor density and binding affinity. In this study comparable low numbers of specific binding sites for ^{3}H-PGE_1, ^{3}H-PGE_2 as well as ^{3}H-iloprost were found on cultured human hypernephroma cells which resemble the results obtained for thyroid cancer (Virgolini *et al.*, 1988) and human hepatocellular cancer (Virgolini *et al.*, 1989). We (Virgolini *et al.*, 1988, 1989) and others (Robertson *et al.*, 1980) proposed a down-regulation mechanism for PG binding sites in malignant tissues which might be due to an increased PG-production by malignant cells (Bennett *et al.*, 1982; Porteder *et al.*, 1984).

Elevated PG production was found in various tumour tissues (Honn *et al.*, 1981; Bennett *et al.*, 1982; Porteder *et al.*, 1984). However, the type of PG produced by tumour cells as well as the capacity of the tumour cells to synthesise PGs are highly variable (Malachi *et al.*, 1981; Ziboh *et al.*, 1981). Cummings and Robertson (1977) have provided evidence for an increased production of PGs by human hypernephroma tumour tissues. They found increased levels of PGA and PGE in cultured hypernephroma cells, in extracts of primary and metastatic hypernephroma tissues and in the venous effluent of a hypernephroma bearing kidney. In accordance with their findings we have now demonstrated that cultured human hypernephroma cells are able to produce various eicosanoids (PGE_2, $PGF_{2\alpha}$, TXB_2, PGD_2 and 12-HETE) with PGE_2 being the main metabolisation product.

For all three PG receptor subtypes investigated we found a linear Scatchard plot indicating a single class of binding sites within the concentration ligand ranges studied. Comparable numbers of binding sites were found for ^{3}H-iloprost and ^{3}H-PGE_1 with a K_d value of about 4 nM for ^{3}H-PGE_1 binding and of 6 nM ^{3}H-iloprost binding on the average. While for

Table I ^{3}H-iloprost binding to cultured human hypernephroma cells

					Without irradiation				*Irradiation*			
					Control (group 1)		*ASA (group 2)*		*Control (group 3)*		*ASA (group 4)*	
Pat.	*Age*	*Sex*	*TNM*	*Stage*	B_{max}	K_d	B_{max}	K_d	B_{max}	K_d	B_{max}	K_d
1	45	m	T3NoMx	II	741	10.0	813	8.7	–	–	–	–
2	78	m	T3NoMx	II	635	12.0	855	10.0	–	–	–	–
3	48	f	T3NoMx	II	876	13.0	987	10.0	332	21.0	489	6.9
4	62	m	T3NoMo	II	732	10.0	960	4.3	287	5.7	354	4.95
5	46	m	T1NoMx	II	808	1.0	916	1.0	–	–	–	–
6	69	m	T3NoMx	III	806	3.6	1047	2.1	432	10.3	867	4.2
7	56	m	T2NoMo	II	649	1.8	2024	1.2	699	1.3	1551	1.1
8	69	m	T3NoMo	I	407	3.2	1301	3.3	–	–	–	–
9	70	f	T2NoMo	II	382	3.6	442	1.8	204	1.5	364	1.9
10	48	m	T3N1Mo	II	829	1.3	1023	1.5	203	6.1	1162	1.9
11	74	m	T3N1Mo	III	756	6.3	861	2.1	118	1.7	576	1.3
$\bar{x}$	61				693	6.0	1021[a]	4.2[b]	325[c]	6.8	766[d]	3.2[b]
± s.d.	± 12				± 164	± 4.5	± 391	± 3.6	± 194	± 7.1	± 452	± 2.2

B_{max}: binding capacity in fmol mg^{-1} protein; K_d: (dissociation constant) in nM; –: not investigated. [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$, *vs* the control group (without irradiation). [d]$P < 0.05$, *vs* the irradiated control group. Significant ($P < 0.05$) increase in ^{3}H-iloprost receptors expressed on hypernephroma cells was found after pre-treatment with ASA which also led to a significant ($P < 0.05$) increase in the binding affinity. Irradiation caused significant ($P < 0.001$) depression of ^{3}H-iloprost receptors while the binding affinity remained unchanged. Irradiated ASA-pretreated cells had a similar binding capacity to the plane control group and a significantly ($P < 0.05$) higher one as compared with the irradiated control group.

most cells ^{3}H-PGE$_1$ as well as ^{3}H-iloprost seem to bind to two binding classes with different affinity (Virgolini *et al.*, 1992), our results on cultured human hypernephroma cells indicate a single class of high affinity sites for either ligand. Furthermore, the competition studies show that ^{3}H-iloprost could recognise ^{3}H-PGE$_1$ sites as does ^{3}H-PGE$_1$ with ^{3}H-iloprost sites vice versa, pointing to a common PGE$_1$/PGE$_2$ binding site on human hypernephroma cells. As with non-malignant cells (Virgolini *et al.*, 1992) ^{3}H-PGE$_2$ did not bind to PGE$_1$/PGE$_2$ binding sites suggesting a distinct class of ^{3}H-PGE$_2$ binding sites.

In order to study the effects of ASA on the PG-system of human hypernephroma cells an incubation was performed for 24 h. ASA significantly depressed PG-production and increased PG receptor densities and binding affinities, whereas cellular viability remained unchanged. These results indicate that ASA renders human hypernephroma cells more sensitive for binding PGE$_1$, PGE$_2$ as well as iloprost. Whether or not there exists a down-regulation mechanism on the basis of an increased PG production by human hypernephroma cells is not yet clear.

Recently a number of studies in murine tumours have shown that indomethacin significantly increases radioresponse of tumours which produce PGs (Furuta *et al.*, 1988*a*, 1988*b*). Initially it was proposed that the augmentation of radioresponse induced by indomethacin could be due to lowering PGs in tumour tissues. The effects of PGs on radioprotection are, however, not clear: some authors reported that PGs might act as radioprotectors (Hanson & Ainsworth, 1985; Walden *et al.*, 1987), whereas others (Milas *et al.*, 1990) proposed an opposite effect.

PGs exert their effects through interaction with specific cell surface receptors. There is little information about PG receptor expressed by tumour cells after radiotherapy. We were now able to show that although the number of PG binding sites was significantly diminished after irradiation with ^{60}Co, no significant change in the number of PG binding sites was found for human hypernephroma cells after pre-incubation with ASA. The results indicate that the PG synthesis inhibitor ASA could protect against an irradiation effect on PG receptors, modifying the radioresponse of human hypernephroma cells.

Table II ^{3}H-PGE$_1$ binding to cultured human hypernephroma cells

	Without irradiation				*Irradiation*			
	Control (group 1)		*ASA (group 2)*		*Control (group 3)*		*ASA (group 4)*	
Pat.	B_{max}	K_d	B_{max}	K_d	B_{max}	K_d	B_{max}	K_d
1	781	10.0	811	5.0	–	–	–	–
3	372	5.0	923	2.9	267	7.1	679	5.0
4	978	2.0	1076	3.0	–	–	–	–
5	911	5.0	1019	5.0	–	–	–	–
6	694	4.8	1367	3.0	504	13.2	867	5.0
7	893	1.3	897	1.5	301	3.2	615	2.4
8	1041	2.7	1773	1.7	–	–	–	–
9	643	1.5	736	1.4	137	1.1	268	1.3
10	555	1.8	1064	1.9	319	3.3	884	3.0
11	685	2.9	1171	2.3	68	1.8	879	1.1
$\bar{x}$	755	3.7	1084[b]	2.8[a]	266[c]	5.0	699[d]	3.0
± s.d.	± 206	± 2.7	303	± 1.3	± 153	± 5.0	± 240	± 1.7

B_{max}: binding capacity in fmol mg^{-1} protein; K_d: (dissociation constant) in nM; –: not investigated. [a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$, *vs* the control group (without irradiation). [d]$P<0.01$, *vs* the irradiated control group. Significant ($P<0.01$) increase in ^{3}H-PGE$_1$ receptors expressed on human hypernephroma cells was found after pre-treatment with ASA which also led to a significant ($P<0.05$) increase in the binding affinity. Irradiation caused a significant ($P<0.001$) depression of ^{3}H-PGE$_1$ receptors without change in the binding affinity. Irradiated ASA-pre-treated cells had a similar binding capacity to the plane control group and a significant ($P<0.01$) higher one as compared with the irradiated control group.

Table III ^{3}H-PGE$_2$ binding to cultured human hypernephroma cells

	Without irradiation				*Irradiation*			
	Control (group 1)		*ASA (group 2)*		*Control (group 3)*		*ASA (group 4)*	
Pat.	B_{max}	K_d	B_{max}	K_d	B_{max}	K_d	B_{max}	K_d
1	563	3.0	650	3.5	–	–	–	–
3	143	5.0	162	1.0	63	4.6	181	2.0
4	478	6.0	858	4.0	–	–	–	–
5	650	2.0	780	2.0	–	–	–	–
6	930	7.7	1104	4.6	209	9.0	336	6.0
8	438	1.0	986	1.2	218	2.2	338	3.0
9	534	1.1	666	1.0	112	1.2	466	1.2
10	313	5.8	324	0.3	96	2.0	232	3.6
11	398	6.3	441	2.3	189	9.1	170	5.0
$\bar{x}$	494	4.2	663[a]	2.2[a]	148[b]	4.7	287[c]	3.5[c]
± s.d.	± 221	± 2.5	309	± 1.5	± 66	± 3.6	± 114	± 1.8

B_{max}: binding capacity in fmol mg^{-1} protein; K_d: (dissociation constant) in nM; –: not investigated. [a]$P<0.05$, [b]$P<0.01$, *vs* the control group (without irradiation). [c]$P<0.05$, *vs* the irradiated control group. Significant ($P<0.05$) increase in ^{3}H-PGE$_2$ receptors expressed on human hypernephroma cells was found after pre-treatment with ASA which also led to a significant ($P<0.05$) increase in the binding affinity. Irradiation caused a significant ($P<0.01$) depression of ^{3}H-PGE$_2$ receptors without change in the binding affinity. Irradiated ASA-pretreated cells had a significantly ($P<0.05$) higher number of ^{3}H-PGE$_2$ receptors as irradiated ASA-untreated cells, however values were still significantly ($P<0.05$) lower than in the control group.

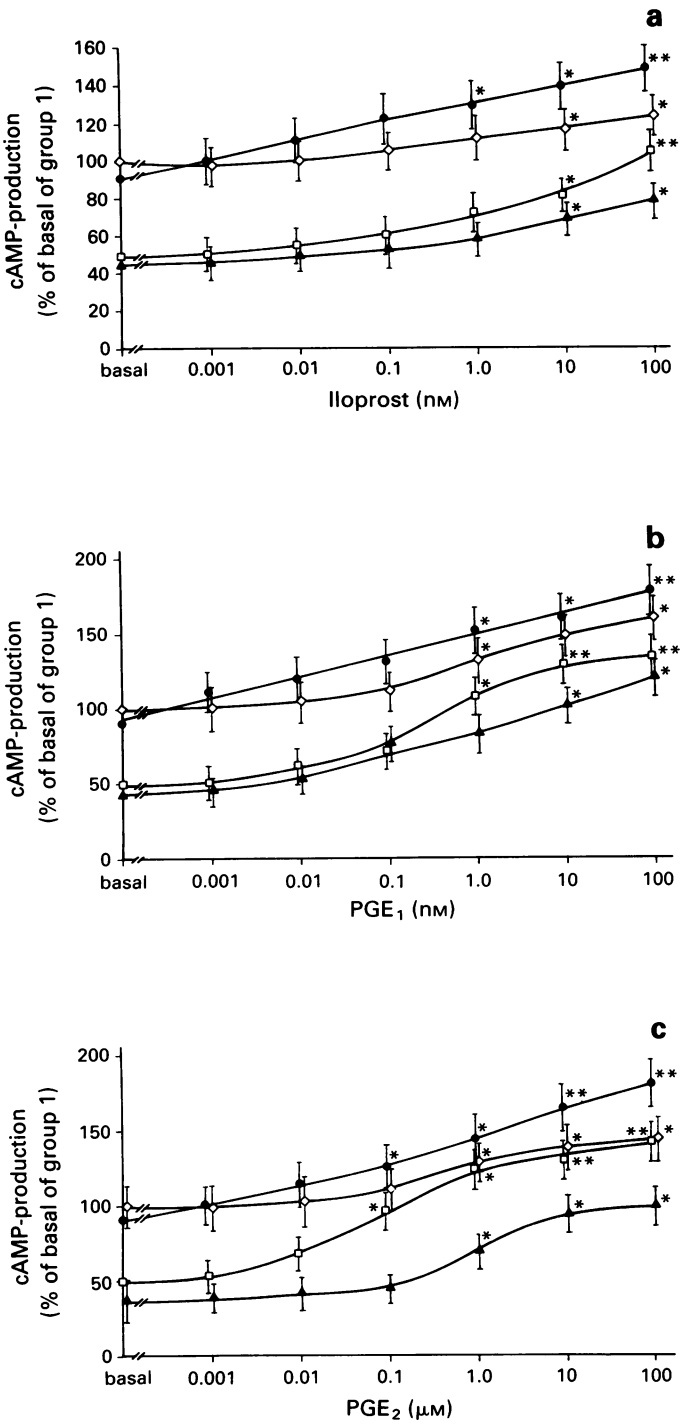

Figure 4 **a–c** Effects of aspirin and irradiation on cAMP-production of hypernephroma cells stimulated by different concentrations of PGs (iloprost **a**, PGE_1 **b**, PGE_2 **c**). Values indicate the means of four independent experiments. –◇–: control group; –●–: ASA-treated group; –▲–: control group irradiated; –□–: ASA-treated group irradiated. $*P<0.05$, $**P<0.01$ *vs* the basal value in each group.

The presence of PGs as well as PG receptors in cultured human hypernephroma cells suggests a role for PGs in tumour growth and possibly its metastases formation. It has already been reported that certain syndromes of patients with hypernephromas such as the osteolytic process and consecutive hypercalcaemia might result from PGs produced by hypernephroma cells (Robertson *et al.*, 1975; Cummings & Robertson, 1977). Furthermore, increased PG production by tumours has been associated with aggression, whereas inhibitors of PG synthesis including ASA and indomethacin were reported to retard tumour growth and metastases formation (Lynch *et al.*, 1978; Bennett, 1982).

Table IV Effects of ASA and irradiation on ^{14}C-AA conversion (in %) by human hypernephroma cells

Pat.	*TXB_2*	*PGE_2*	*PGD_2*	*$PGF_{2\alpha}$*	*12-HETE*	*AA (%)*
			Control group (group 1)			
1	8.5	12.3	2.2	2.3	4.2	65.2
3	6.2	11.0	2.1	2.4	3.8	58.9
4	11.8	20.1	3.1	3.9	4.5	49.3
6	7.3	7.9	3.9	4.2	3.1	68.9
8	5.9	9.8	4.7	2.9	4.8	62.9
9	8.9	15.3	5.1	3.8	3.1	59.8
11	7.7	7.5	3.9	2.9	2.1	67.8
$\bar{x}$ ± s.d.	8.0 ± 2.0	12.0 ± 4.5	3.6 ± 3.6	3.2 ± 0.8	3.7 ± 1.0	61.8 ± 6.7
			ASA-treated group (group 2)			
1	1.2	1.8	1.3	1.7	6.9	66.5
3	0.8	1.1	0.9	0.7	4.2	78.2
4	1.7	2.2	0.2	0.8	7.3	72.9
6	1.3	0.7	0.5	0.2	5.5	78.5
8	1.3	1.5	0.4	0.3	5.3	82.8
9	2.3	1.8	0.6	0.5	4.9	81.0
11	1.5	1.3	0.8	0.3	8.8	78.5
$\bar{x}$ ± s.d.	$1.4^{c,e}$ ± 0.5	$1.5^{c,e}$ ± 0.5	$0.7^{b,e}$ ± 0.4	$0.6^{c,e}$ ± 0.5	$6.1^{a,d}$ ± 1.6	76.9^{b} ± 5.5
			Control group irradiated (group 3)			
1	6.6	8.3	2.9	2.1	4.6	70.4
4	8.2	9.3	3.1	3.9	5.2	63.2
6	6.9	7.8	3.3	2.5	4.3	68.9
9	9.1	10.5	2.9	2.6	3.6	65.2
11	6.4	9.3	2.9	2.4	2.8	67.5
$\bar{x}$ ± s.d.	7.4 ± 1.2	9.0 ± 1.0	3.0 ± 0.2	2.7 ± 0.7	4.1 ± 0.9	67.0 ± 2.9
			ASA-treated group irradiated (group 4)			
3	1.3	0.8	0.3	0.5	4.9	82.9
4	1.2	1.6	0.9	0.9	5.1	74.3
6	1.1	1.9	0.8	0.2	4.8	81.2
9	1.5	1.1	0.5	0.8	4.8	73.7
11	0.6	1.8	0.4	0.4	5.1	79.4
$\bar{x}$ ± s.d.	$1.1^{c,f}$ ± 0.3	$1.4^{c,f}$ ± 0.5	0.6^{f} ± 0.3	$0.6^{c,f}$ ± 0.3	4.9^{a} ± 0.2	$78.3^{c,e}$ ± 4.1

$^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$, *vs* the control group (group 1). $^{d}P<0.05$, $^{e}P<0.01$, $^{f}P<0.001$, *vs* the irradiated control group.

cAMP is a second messenger mediating the actions of PGs and also connected with normal and malignant cell proliferation. It is likely that changes in intracellular cAMP-production due to PG stimulation may greatly influence tumour cell growth and even metastases formation (Heidrick & Ryan, 1971; Sheppard, 1972). However, both reduced cAMP levels (Malachi *et al.*, 1981; Goldberg *et al.*, 1975; Stevens *et al.*, 1978) and elevated cAMP contents (Minton *et al.*, 1976; Küng *et al.*, 1977) have been reported in tumour tissues. This may correlate to the different PG levels found for various tumour tissues. For cultured human hypernephroma cells we found no significant difference in basal cAMP levels in both non-ASA-treated and ASA-treated cells. However, the PG-stimulated cAMP-production was significantly higher in the ASA-treated group as compared with the control group. This suggests that down-regulation of PG receptors was associated with decreased adenylate cyclase activity stimulated by PGs.

We here show that ASA modifies the radioresponse of human hypernephroma cells by preventing the irradiation-induced decrease of PG receptors, which might cause a corresponding decrease in PG-induced membrane adenylate cyclase activity.

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