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 PGE₁, PGE₂ as well as PGI₂-binding sites (PGE₁: \( B_{\text{max}} = 755 \pm 206 \text{fmol mg}^{-1} \); protein, \( K_d = 3.7 \pm 0.7\text{nM} \); PGE₂: \( B_{\text{max}} = 494 \pm 221 \text{fmol mg}^{-1} \); protein, \( K_d = 4.2 \pm 2.5\text{nM} \); PGI₂: \( B_{\text{max}} = 693 \pm 164 \text{fmol mg}^{-1} \); protein, \( K_d = 6.0 \pm 4.5\text{nM} \)). Significant \((P<0.01)\) increase in PG binding sites expressed on human hypernephroma cells (PGE₁: \( B_{\text{max}} = 1084 \pm 303 \text{fmol mg}^{-1} \); protein, \( K_d = 2.8 \pm 1.3\text{nM} \); PGE₂: \( B_{\text{max}} = 663 \pm 309 \text{fmol mg}^{-1} \); protein, \( K_d = 2.2 \pm 1.5\text{nM} \); PGI₂: \( B_{\text{max}} = 1021 \pm 391 \text{fmol protein}, K_d = 4.2 \pm 3.6\text{nM} \)) and inhibition of PG biosynthesis (TXB₂: \(-82.5\\%\); PGE₁: \(-87.5\\%\); PGI₂: \(-80.6\\%\); PGI₂: \(-81.3\\%\)) were found after acetylsalicylic acid (ASA)-treatment (0.5 \( \mu \text{g ml}^{-1} \) cells for 24 h). Following irradiation (\( ^{60}\text{Co}, 1.0 \text{Gy min}^{-1} \) over 10 min), PG binding sites (PGE₁: \( B_{\text{max}} = 266 \pm 153 \text{fmol mg}^{-1} \); protein, \( K_d = 5.0 \pm 5.0\text{nM} \); PGE₂: \( B_{\text{max}} = 148 \pm 66 \text{fmol mg}^{-1} \); protein, \( K_d = 4.7 \pm 3.6\text{nM} \); PGI₂: \( B_{\text{max}} = 325 \pm 194 \text{fmol mg}^{-1} \); protein, \( K_d = 6.8 \pm 7.1\text{nM} \)) were significantly \((P<0.01)\) diminished. However, irradiation had no significant effect on PG bindig sites in ASA-pretreated cells (PGE₁: \( B_{\text{max}} = 699 \pm 240 \text{fmol mg}^{-1} \); protein, \( K_d = 3.5 \pm 1.8\text{nM} \); iloprost: \( B_{\text{max}} = 766 \pm 452 \text{fmol mg}^{-1} \); protein, \( K_d = 3.2 \pm 2.2\text{nM} \)). Although there was no significant difference in the basal values for CAMP between control and ASA-treated 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 chemotherapeutics (Powles et al., 1978), immuno- (Chun & Hoffman, 1987) and/or radiotherapy (Furuta et al., 1988a) 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., 1988a). Furthermore, the response to indomethacin treatment was dependent on the ability of the tumour to produce PGs, mainly PGE₁ and PGI₂ (Furuta et al., 1988b). Apart from the fact that the mechanisms by which indomethacin potentiates tumour radioresistance 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 radioreponse 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 seem 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 cm³) 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% CO₂, 97.5% O₂ 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 iU 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
testing the multiplicity of cells with different concentrations of Cytochalasin B (CB) and one part was plated into plastic culture chambers (Lab-Tek), fixed after 24–72 h and checked for content of cytokeratin as an indicator of malignancy (Dierck 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·mL⁻¹ medium for 24 h);
3. Control group irradiated: irradiation was performed with gamma rays from a 60Co unit (Gammatron, Siemens) with a source-source 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 (4×10⁵ cells in each group) were washed twice in assay buffer containing 50 mM Tris-HCl (pH 7.5), 5 mM MgCl₂, 1 mM CaCl₂, 0.1 M NaCl and centrifuged at 500g 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 (Comassie 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 [³H]-iloprost (a chemically stable PG₁-analog (Skuballa & Vorbrüggen, 1983); specific activity 14.7 Ci mmol⁻¹; radiochemical purity 98.7%; 0.5–100 nM), [³H]-PGE₁ (specific activity 42 Ci mmol⁻¹; radiochemical purity 97.5%; 0.5–100 nM) or [³H]-PGE₂ (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 [³H]-iloprost, [³H]-PGE₁, or [³H]-PGE₂ 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 [³H]-PGE₁, [³H]-PGE₂ or [³H]-iloprost in the absence (B) 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).

[³H]-iloprost, [³H]-PGE₁, [³H]-PGE₂, and iloprost were obtained from Amersham International, Buckinghamshire, UK. Unlabelled PGE, and PGE₂ 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 6×10⁵ 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 PGE₁, PGE₂, or iloprost in a concentration range from 10⁻⁰ M to 10⁻³ M. After 30 min the cells were homogenised by ultrasond (Sonicator, W-220F, Plainview, NY) and ultratraps (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 [¹²⁵I]-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 variability was 5.6 ± 2.3%.

**Radiothinlayer chromatography**

The cells (4–6×10⁵ in each group) were resuspended in 50 mM Tris-HCl buffer (pH 7.5) and incubated with 0.25 μg 4-Carachidonic 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](image_url)  
**Figure 1** Time course of specific [³H]-PG binding to human hypernephroma cells. Association: [³H]-iloprost (*), [³H]-PGE₁ (○) (each 84 nm) or [³H]-PGE₂ (△) (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. [³H]-PGs were displaceable 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, Hilten-Syringe, Hamilton, Bonaduz, Switzerland) and placed onto a silica gel plate (Merck) together with the standards (PGE\textsubscript{1}, PGE\textsubscript{2}, 12-HETE, TXB\textsubscript{2}, PGD\textsubscript{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\textsubscript{2}O 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 (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 \( ^3\text{H}-\text{iloprost}, ^3\text{H}-\text{PGE}_1 \) and \( ^3\text{H}-\text{PGE}_2 \) to cultured human hypernephroma cells

In initial experiments the time course of \( ^3\text{H}-\text{iloprost}, ^3\text{H}-\text{PGE}_1 \) and \( ^3\text{H}-\text{PGE}_2 \)-binding to human hypernephroma cells was investigated showing a constant increase of \( ^3\text{H} \)-ligand specifically bound during the first min of incubation (Figure 1). Equilibrium was reached within 20 min in \( ^3\text{H}-\text{iloprost} \) and \( ^3\text{H}-\text{PGE}_2 \) binding experiments and within 30 min in \( ^3\text{H}-\text{PGE}_2 \) 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 \( ^3\text{H}-\text{iloprost}, ^3\text{H}-\text{PGE}_1 \) and \( ^3\text{H}-\text{PGE}_2 \) binding was only slightly dependent on temperature and was insignificantly lower at 37°C and at 22°C than at 4°C. \( ^3\text{H}-\text{iloprost}, ^3\text{H}-\text{PGE}_1 \) and \( ^3\text{H}-\text{PGE}_2 \)-binding to human hypernephroma cells could be displaced by unlabelled iloprost, PGE\textsubscript{1}, and PGE\textsubscript{2}, respectively. In \( ^3\text{H}-\text{iloprost} \)-binding studies the concentrations of unlabelled iloprost, PGE\textsubscript{1}, PGE\textsubscript{2} for causing half maximal inhibition (IC\textsubscript{50}) were 81 ± 25 \( \times 10^{-6} \) M, 12 ± 6 \( \times 10^{-8} \) M and 9 ± 3 \( \times 10^{-8} \) M, respectively. The IC\textsubscript{50} values were 40 ± 10 \( \times 10^{-9} \) M for PGE\textsubscript{1}, 86 ± 20 \( \times 10^{-9} \) M for iloprost, and 85 ± 26 \( \times 10^{-9} \) M for PGE\textsubscript{2} in \( ^3\text{H}-\text{PGE}_2 \) binding experiments, and amounted to 42 ± 9 \( \times 10^{-9} \) M for PGE\textsubscript{2}, 4 ± 2 \( \times 10^{-9} \) M for PGE\textsubscript{1} and 30 ± 7 \( \times 10^{-9} \) M for iloprost in \( ^3\text{H}-\text{PGE}_2 \) binding studies (Figure 2a–c).

Saturation of \( ^3\text{H}-\text{PG} \)-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 \( ^3\text{H}-\text{iloprost} \)-receptors from 693 ± 164 to 1021 ± 391 fmol mg\(^{-1}\) protein (\( P<0.05 \)), \( ^3\text{H}-\text{PGE}_1 \)-receptors from 755 ± 206 to 1084 ± 303 fmol mg\(^{-1}\) protein (\( P<0.01 \)) and \( ^3\text{H}-\text{PGE}_2 \)-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 46Co 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 significant decrease in \( ^3\text{H}-\text{PG} \)-receptors: \( ^3\text{H}-\text{iloprost} \) receptor decreased to 325 ± 194 fmol mg\(^{-1}\) protein (\( P<0.001 \)), \( ^3\text{H}-\text{PGE}_1 \)-receptors to 266 ± 153 fmol mg\(^{-1}\) protein (\( P<0.001 \)), and \( ^3\text{H}-\text{PGE}_2 \)-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 (\( P>0.05 \)) (\( ^3\text{H}-\text{iloprost} \) receptors: 766 ± 452 fmol mg\(^{-1}\) protein, \( ^3\text{H}-\text{PGE}_1 \)-receptors: 699 ± 240 fmol mg\(^{-1}\) protein, \( ^3\text{H}-\text{PGE}_2 \)-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\(^{-1}\) protein) 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\textsubscript{1} and PGE\textsubscript{2} significantly (\( P<0.01 \)) stimulated cAMP-production in all four groups dose-dependently. However, the half maximal effective doses (ED\textsubscript{50})
were significantly different between the four groups (Figure 4a–c). The corresponding ED₅₀ 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 $43 \pm 11 \times 10^{-3}$ M for the ASA-treated group irradiated. The ED₅₀ for PGE₂ 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 ED₅₀ values for PGE₁ were $11 \pm 4 \times 10^{-7}$ 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.

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

Hypernephroma cells converted exogeneous precursor AA to a various number of compounds (HETE, PGD₂, thromboxane B₂, TXB₂, PGE₂, PGF₆α) (Table IV). The main metabolites of AA conversion of cultured hypernephroma cells were PGE₂ and TXB₂ 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 $^{3}H$-PGE₁, $^{3}H$-PGE₂ 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; Porter et al., 1984).

Elevated PG production was found in various tumour tissues (Honn et al., 1981; Bennett et al., 1982; Porter 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 (PGE₂, PGF₂α, TXB₂, PGD₂ and 12-HETE) with PGE₂ being the main metabolism 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 $^{3}H$-PGE₁ with a $K_d$ value of about 4 nM for $^{3}H$-PGE binding and 6 nM $^{3}H$-iloprost binding on the average. While for

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Table 1 $^{3}H$-iloprost binding to cultured human hypernephroma cells

| Pat. | Age | Sex | TNM | Stage | B₅₀ (group 1) | B₅₀ (group 2) | B₅₀ (group 3) | B₅₀ (group 4) |
|------|-----|-----|-----|-------|-------------|-------------|-------------|-------------|
| 1    | 45  | m   | T3N0M | II   | 741         | 10.0        | 813         | 8.7         |
| 2    | 78  | m   | T3N0M | II   | 635         | 12.0        | 855         | 10.0        |
| 3    | 48  | f   | T3N0M | II   | 876         | 13.0        | 987         | 10.0        |
| 4    | 62  | m   | T3N0M | II   | 732         | 10.0        | 960         | 4.3         |
| 5    | 46  | m   | T1N0M | II   | 808         | 1.0         | 916         | 1.0         |
| 6    | 69  | m   | T3N0M | III  | 806         | 3.6         | 1047        | 2.1         |
| 7    | 56  | m   | T2N0M | II   | 649         | 1.8         | 2024        | 1.2         |
| 8    | 69  | m   | T3N0M | II   | 407         | 3.2         | 1301        | 3.3         |
| 9    | 70  | f   | T2N0M | II   | 382         | 3.6         | 442         | 1.8         |
| 10   | 48  | m   | T3N1M | II   | 829         | 1.3         | 1023        | 1.5         |
| 11   | 74  | m   | T3N1M | III  | 756         | 6.3         | 861         | 2.1         |
| x    | 61  |     |      |      | 693         | 6.0         | 1021        | 4.2         |

B₅₀: binding capacity in fmol mg⁻¹ protein; K_d (dissociation constant) in nM; -- not investigated. *P < 0.05, **P < 0.01, ***P < 0.001, vs the control group (without irradiation). $^{a}$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 ³H-PGE₁ as well as ³H-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 ³H-iloprost could recognise ³H-PGE₁ sites as does ³H-PGE₂ with ³H-iloprost sites vice versa, pointing to a common PGE₁/PGE₂ binding site on human hypernephroma cells. As with non-malignant cells (Virgolini et al., 1992) ²H-PGE₂ did not bind to PGE₁/PGE₂ binding sites suggesting a distinct class of ³H-PGE₂ 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 PGE₁, PGE₂ 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 radiosponse of tumours which produce PGs (Furuta et al., 1988a, 1988b). Initially it was proposed that the augmentation of radiosresponse 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 (Mias 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 ⁶⁰Co, 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 radiosresponse of human hypernephroma cells.

**Table II ³H-PGE₁ binding to cultured human hypernephroma cells**

| Pat. | Without irradiation | Irradiation |
|------|---------------------|-------------|
|      | Control (group 1)   | **ASA** (group 2) | **Control** (group 3) | **ASA** (group 4) |
|      | B<sub>max</sub> | K<sub>d</sub> | B<sub>max</sub> | K<sub>d</sub> | B<sub>max</sub> | K<sub>d</sub> | B<sub>max</sub> | K<sub>d</sub> |
| 1    | 781 ± 2.7           | 50 ± 1.3    | 267 ± 0.5 | 50 ± 1.3 | 679 ± 0.5 | 5.0 |
| 3    | 50 ± 1.3            | 303 ± 1.3   | 50 ± 1.3 | 303 ± 1.3 | 50 ± 1.3 | 5.0 |
| 4    | 50 ± 1.3            | 50 ± 1.3    | 50 ± 1.3 | 50 ± 1.3 | 50 ± 1.3 | 5.0 |
| 5    | 50 ± 1.3            | 50 ± 1.3    | 50 ± 1.3 | 50 ± 1.3 | 50 ± 1.3 | 5.0 |
| 6    | 50 ± 1.3            | 50 ± 1.3    | 50 ± 1.3 | 50 ± 1.3 | 50 ± 1.3 | 5.0 |
| 7    | 50 ± 1.3            | 50 ± 1.3    | 50 ± 1.3 | 50 ± 1.3 | 50 ± 1.3 | 5.0 |
| 8    | 50 ± 1.3            | 50 ± 1.3    | 50 ± 1.3 | 50 ± 1.3 | 50 ± 1.3 | 5.0 |
| 9    | 50 ± 1.3            | 50 ± 1.3    | 50 ± 1.3 | 50 ± 1.3 | 50 ± 1.3 | 5.0 |
| 10   | 50 ± 1.3            | 50 ± 1.3    | 50 ± 1.3 | 50 ± 1.3 | 50 ± 1.3 | 5.0 |
| 11   | 50 ± 1.3            | 50 ± 1.3    | 50 ± 1.3 | 50 ± 1.3 | 50 ± 1.3 | 5.0 |

**Table III ³H-PGE₂ binding to cultured human hypernephroma cells**

| Pat. | Without irradiation | Irradiation |
|------|---------------------|-------------|
|      | Control (group 1)   | **ASA** (group 2) | **Control** (group 3) | **ASA** (group 4) |
|      | B<sub>max</sub> | K<sub>d</sub> | B<sub>max</sub> | K<sub>d</sub> | B<sub>max</sub> | K<sub>d</sub> | B<sub>max</sub> | K<sub>d</sub> |
| 1    | 563 ± 2.1           | 3.0 ± 2.1   | 63 ± 4.6 | 181 ± 2.0 | 63 ± 4.6 | 181 ± 2.0 | 63 ± 4.6 | 181 ± 2.0 |
| 3    | 143 ± 2.1           | 5.0 ± 2.1   | 63 ± 4.6 | 181 ± 2.0 | 63 ± 4.6 | 181 ± 2.0 | 63 ± 4.6 | 181 ± 2.0 |
| 4    | 478 ± 2.1           | 6.0 ± 2.1   | 63 ± 4.6 | 181 ± 2.0 | 63 ± 4.6 | 181 ± 2.0 | 63 ± 4.6 | 181 ± 2.0 |
| 5    | 780 ± 2.1           | 858 ± 2.1   | 63 ± 4.6 | 181 ± 2.0 | 63 ± 4.6 | 181 ± 2.0 | 63 ± 4.6 | 181 ± 2.0 |
| 6    | 930 ± 2.1           | 1104 ± 2.1  | 63 ± 4.6 | 181 ± 2.0 | 63 ± 4.6 | 181 ± 2.0 | 63 ± 4.6 | 181 ± 2.0 |
| 8    | 438 ± 2.1           | 986 ± 2.1   | 63 ± 4.6 | 181 ± 2.0 | 63 ± 4.6 | 181 ± 2.0 | 63 ± 4.6 | 181 ± 2.0 |
| 9    | 534 ± 2.1           | 666 ± 2.1   | 63 ± 4.6 | 181 ± 2.0 | 63 ± 4.6 | 181 ± 2.0 | 63 ± 4.6 | 181 ± 2.0 |
| 10   | 313 ± 2.1           | 324 ± 2.1   | 63 ± 4.6 | 181 ± 2.0 | 63 ± 4.6 | 181 ± 2.0 | 63 ± 4.6 | 181 ± 2.0 |
| 11   | 398 ± 2.1           | 441 ± 2.1   | 63 ± 4.6 | 181 ± 2.0 | 63 ± 4.6 | 181 ± 2.0 | 63 ± 4.6 | 181 ± 2.0 |

B<sub>max</sub>: binding capacity in fmol mg⁻¹ protein; K<sub>d</sub>: dissociation constant in nM; ±: not investigated. <sup>a</sup>P < 0.05, <sup>b</sup>P < 0.01, <sup>c</sup>P < 0.001, vs the control group (without irradiation). <sup>d</sup>P < 0.01, vs the irradiated control group. Significant (P < 0.01) increase in ³H-PGE₁ 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 ³H-PGE₁ 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.
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 ¹⁴C-AA conversion (in %) by human hypernephroma cells

| Pat. | TXB₂ | PGE₁ | PGD₂ | PGE₃ | 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 |
| X ± s.d. | 8.0 | 12.0 | 3.6 | 3.2 | 3.7 | 61.8 |

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 |
| X ± s.d. | 1.4<sup> a </sup> | 1.5<sup> a </sup> | 0.7<sup> a </sup> | 0.6<sup> a </sup> | 6.1<sup> a </sup> | 76.9<sup> a </sup> |

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 |
| X ± s.d. | 7.4 | 9.0 | 3.0 | 2.7 | 4.1 | 67.0 |

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 |
| X ± s.d. | 1.1<sup> c,f </sup> | 1.4<sup> c,f </sup> | 0.6<sup> c,f </sup> | 0.6<sup> c,f </sup> | 4.9<sup> c,b </sup> | 78.3<sup> c,e </sup> |

### 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 radioreponse 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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