Eur J Cancer, Vol. 26, No. 7, pp. 802–807, 1990.
Printed in Great Britain

0277–5379/90$3.00 + 0.00
Pergamon Press plc

Specialist Interest Articles

Effects of Prostaglandin and Leukotriene Inhibitors on the Growth of Human Glioma Spheroids

Istvàn Gàti, Mats Bergström, Göran Westerberg, Katalin Csòka, Carin Muhr and Jörgen Carlsson

Established cell-lines of human glioma origin were cultured as multicellular spheroids or as monolayers. Volume growth and ^{3}H-thymidine labelling were analysed for the spheroids after continuous exposure to drugs interfering with the release of arachidonic acid and the production of prostaglandins and leukotrienes. Comparative measurements were made on monolayer cultures. The cyclo-oxygenase inhibitor indomethacin enhanced growth at intermediate concentrations (0.5–5.0 μg/ml) but reduced growth at 50 μg/ml. The dual cyclo-oxygenase and lipoxygenase inhibitor ketoprofen had a significant inhibitory effect on growth and cell proliferation of spheroids at high concentration (50 μg/ml). The weak lipoxygenase inhibitor NDGA (quinone-form) decreased growth whereas the strong lipoxygenase inhibitor NDGA (hydroquinone-form) did not reduce growth rate but significantly decreased cell proliferation. Quinacrine reduced the spheroid growth rate although dexamethasone had no effects. Thus, inhibitors of the arachidonic acid cascade had growth inhibitory effects in the spheroid tumour model as well as in cells cultured as monolayers.
Eur J Cancer, Vol. 26, No. 7, pp. 802—807, 1990.

INTRODUCTION

THE ARACHIDONIC acid cascade may influence the growth of malignant tumours and have a role in host defence against neoplastic transformation [1, 2]. Prostaglandin levels are increased in human and animal tumour models [2, 3]. Cultured tumour cells can produce large amounts of prostaglandins [4–6]. Inhibition of prostaglandin synthesis significantly retards tumour growth in animal models [2, 7–12]. The interrelation between different arachidonic acid metabolites and the possible exchange of metabolites between lymphocytes, macrophages and tumour cells is intriguing [2, 13, 14]. *In vivo*, the effects of prostaglandin and leukotriene inhibitors varies for cells at different stages of differentiation and is modulated by the cellular microenvironments [15].

In tumour cell spheroids, effects related to growth in three dimensions can be studied [16, 17]. The spheroids develop a central necrosis during growth, probably as an effect of limitation in nutrition supply and metabolic gradients in pH and oxygen partial pressure [17–20]. In this system biochemical 'stress' may be induced by substances released from the necrosis, thereby inducing release of arachidonic acid from phospholipids in the cell membrane through the actions of phospholipases. Cell damage, inflammation, and tissue destruction may initiate the arachidonic acid cascade [14, 15].

We have examined the effects of different inhibitors of the arachidonic acid release, as well as inhibitors of the synthesis of prostaglandins and inhibitors of the leukotriene synthesis in spheroids grown from three different cell lines of human glioma origin.

MATERIALS AND METHODS

Cell culture

The human glioma cell lines, U-118 MG, U-343 MGa,$Cl_{2,6}$ (called U-343MGa), and U-251 MG, were originally established by Westermark *et al.* [21]. All cells were cultured in Ham's F-10 medium with 10% fetal bovine serum, L-glutamine (2 nmol/l), penicillin (100 IU/ml), and streptomycin (100 mg/ml)(Flow). The medium was changed three times weekly in all studies. The cultures were kept at 37°C in 5% CO_2, pH 7.3.

Spheroid culture

Monolayer cultures were trypsinized and the cells were placed in spinner flasks with 125 ml medium. The cells were allowed to form and grow as spheroids for 2–3 weeks before the experiments. After the aggregation and the initial growth phase in the spinner flasks, spheroids with similar size (diameter 300–500 μm) were selected and transferred to agarose-coated mini-dishes for liquid overlay culture [22].

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Drugs

Dexamethasone was used as a putative phospholipase-A_2 inhibitor at 0.1, 1.0, 10, and 100 μmol/l. The phospholipase-A_2 inhibition requires corticosteroid receptors and synthesis of an inhibitory protein, lipocortin [23]. The phospholipase-A_2 inhibitor quinacrine was used at the same concentrations as dexamethasone. Indomethacin was used for cyclo-oxygenase inhition at 0.05, 0.5, and 50 μg/ml, and NDGA for lipoxygenase inhibition at doses 0.1, 1, 10, and 100 μmol/l. NDGA was used both in the hydroquinone and quinone form [24]. Ketoprofen, which is a dual cyclo-oxygenase and lipoxygenase inhibitor [8] was used at 0.5, 5, 50 μg/ml. All chemicals were purchased from Sigma.

Drug exposure

The spheroids were placed in non-cell-adhesive, agarose-coated multidishes, each spheroid in its separate well of 0.5 ml. Culture medium with the selected concentrations of drugs were changed three times per week and growth was followed up for 14 days. The spheroids were continuously exposed to the drugs during this time. For hydroquinone-NDGA, to avoid complete drug oxidation, the solution was freshly prepared and added every day.

Five to twelve spheroids were used in each experiment at each drug concentration. At the beginning of the experiment, the diameter differences were less than 100 μm between individual spheroids. The spheroids were randomly divided for drug and control culture. Each experiment was repeated three times and performed on at least two different cell lines. Monolayer culture was compared for indomethacin, NDGA and ketoprofen on U-343MGa cells.

Evaluation of drug effects

Individual spheroids were measured with an ocular scale, manipulated by a micrometer screw, in an inverted Olympus microscope three times per week. The volume (V) of each spheroid was calculated from $V = 4/3\ \pi\ (ab)^{1.5}$, where a and b are minimum and maximum radii measured at right angles. Relative volume of each spheroid was determined by volume obtained during the treatment related to volume measured at the beginning of experiment. Mean values of relative volumes of different spheroid groups were plotted and compared. Differences between the treated and control spheroid groups were evaluated by paired t test.

After 14 days in culture, randomly selected spheroids were incubated for 20 min with 10 μCi/ml ^{3}H-thymidine but without drugs. All spheroids were embedded in methacrylate and cut into 2 μm sections [25]. The sections through the centre of the spheroids were selected for further analysis. Spheroids labelled with ^{3}H-thymidine were processed for autoradiography [26]. Regions were selected 0–100 and 100–200 μm from the spheroid surface in which the labelling index was calculated as the percentage of cells labelled with ^{3}H-thymidine. The means and standard deviations of five spheroids of different groups were calculated, and significance was compared to controls with a paired t test.

Monolayer cultures

Cells from the cell line U-343MGa were cultured as monolayers with conventional techniques and continuously exposed to the different compounds. Each monolayer culture was done in triplicate. The same number of cells were plated at the beginning of the experiments for drug exposures and for controls. The number of cells were counted twice per experiment by Coulter counter. At the first count the cells were trypsinised, the treated cells resuspended in drug-containing medium and the untreated cells in normal medium. Small volumes of suspensions were used for counting. After counting, the same number of cells in all groups as at the beginning of the experiments were replated with drug-containing medium for the treated groups and normal medium for the controls, to make the appropriate dilutions. At the second trypsinisation and counting, growth curves were constructed as if all cells were recovered.

RESULTS

Effects on spheroid volume growth

Figure 1 shows the effects of dexamethasone and quinacrine on the growth patterns of U-118MG and U-343MGa spheroids. Although during dexamethasone treatment some variations were seen between the groups, the graphs of treated groups were close to control and did not always show the same pattern. Quinacrine was inhibitory at 0.1 μmol/l and reduced spheroid size at 10 and 100 μmol/l; under which conditions the spheroids became dark and lost cells from the surface by the third day of treatment. Indomethacin increased growth rate at 0.5 and 5 μg/ml while at the highest dose, 50 μg/ml, an inhibitory effect was seen (Fig. 2). Quinone-NDGA inhibited growth at high dose (100 μmol/l). Figure 3a shows the effects of quinone-NDGA on U-343MGa spheroids. The hydroquinone form increased growth, as shown for U-118MG spheroids in Fig. 3b. Ketoprofen inhibited growth at the highest dose 50 μg/ml (Fig. 4).

The effects of these drugs on spheroid volume growth were not strong but the differences after quinone–NDGA and ketoprofen were significant compared with controls.

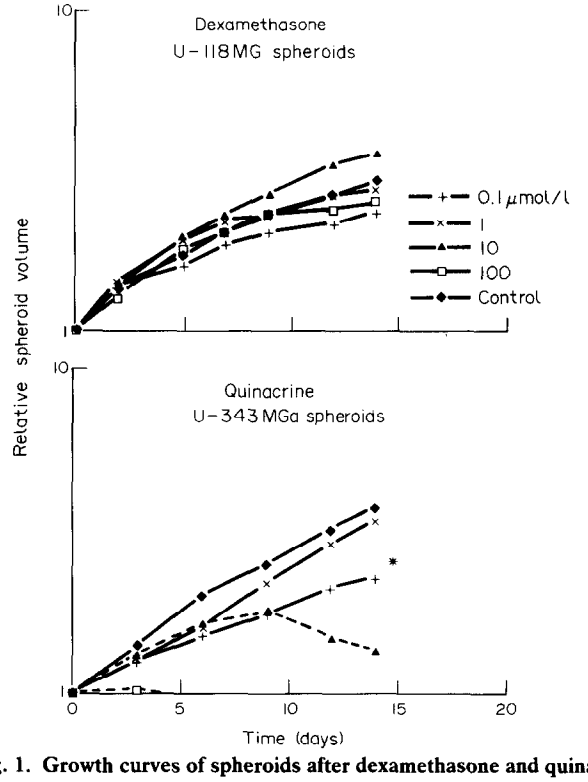

Fig. 1. Growth curves of spheroids after dexamethasone and quinacrine treatment. S.D.s were less than 30% of mean values. Significant differences were shown compared with controls ($P \leq 0.05$). Broken lines indicate measurements disturbed by shedding of cells from spheroid surfaces.

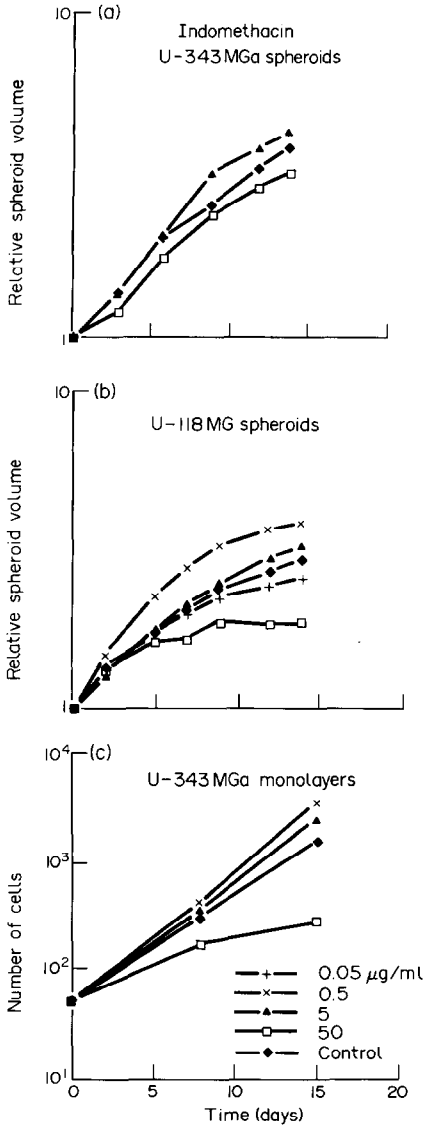

Fig. 2. Growth curves for indomethacin-treated U-343 MGa spheroids (a), U-118MG spheroids (b), and U-343MGa monolayers (c). S.D.s were less than 30% and 40% of mean values in each point of (a) and (b), respectively.

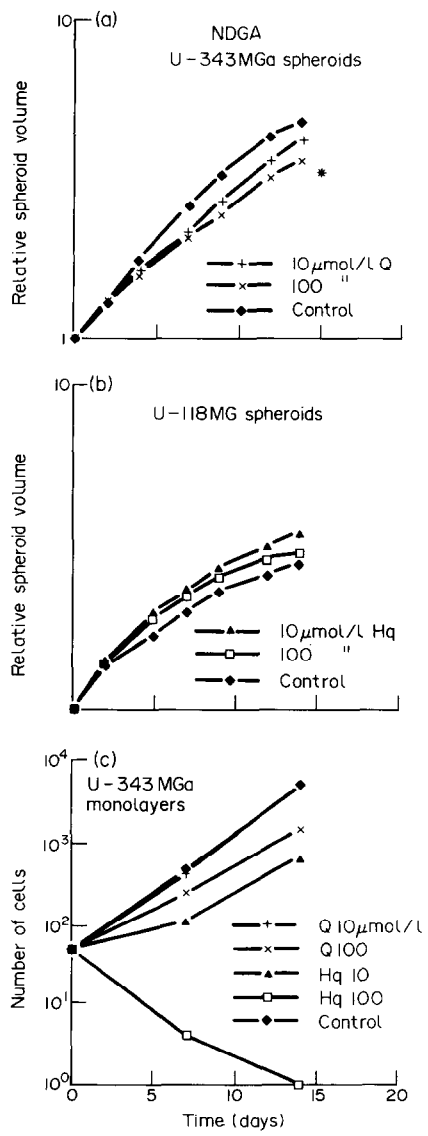

Fig. 3. Growth curves for monolayers and spheroids after NDGA. Q = quinone (a) and Hq = hydroquinone (b) form. S.D.s were less than 30% of mean values in each point. (No. of spheroids showing significantly different curves: 12 control, 11 treated.)

Effects on cell proliferation in spheroids

With dexamethasone, no consistent effects were seen on cell proliferation (data not shown). Labelling indices after exposure to two representative drug concentrations are shown in Table 1 in one cell line for the other drugs. Despite the growth inhibitory effect of 0.1 μmol/l quinacrine no decrease in the labelling index was found; there was even a slight increase in the outer portion of the spheroids. There were no differences after indomethacin exposure. Growth was stimulated at intermediate doses, which might mean that the labelling index increased in the deeper region. Quinone–NDGA decreased the labelling index in the deeper region while hydroquinone-NDGA decreased the index throughout the spheroids. Morphological changes also occurred (swelling, loosening of cell-to-cell contacts), which probably explain the lack of volume growth (Fig. 5). Ketoprofen decreased the labelling index at the peripheral region of U-251MG spheroids, whereas the index in the deeper region increased. The changes in the labelling indices were significant only after NDGA (both forms) and ketoprofen treatments.

Effects on monolayers

Comparative studies of drug effects were made from growth curves of U-343MGa monolayer cells. Ketoprofen and NDGA inhibited the growth of monolayer cultures, except at the lowest concentrations, and the effect increased with dose. At the highest concentrations the number of cells decreased with time. Indomethacin stimulated monolayer growth at the lower doses whereas the highest concentration decreased the growth rate.

DISCUSSION

We compared monolayer and spheroid cultures because the development of an extensive extracellular matrix and the compact three-dimensional growth in spheroids might limit the access of drugs in the deeper regions. In addition, the nutritive conditions and the exchange between adjacent cells of factors influencing growth might be different.

In vivo dexamethasone significantly altered the characteristics of gliomas with a reduction of vascular permeability, a decrease in peritumoral oedema, and a diminished tumour volume [27,

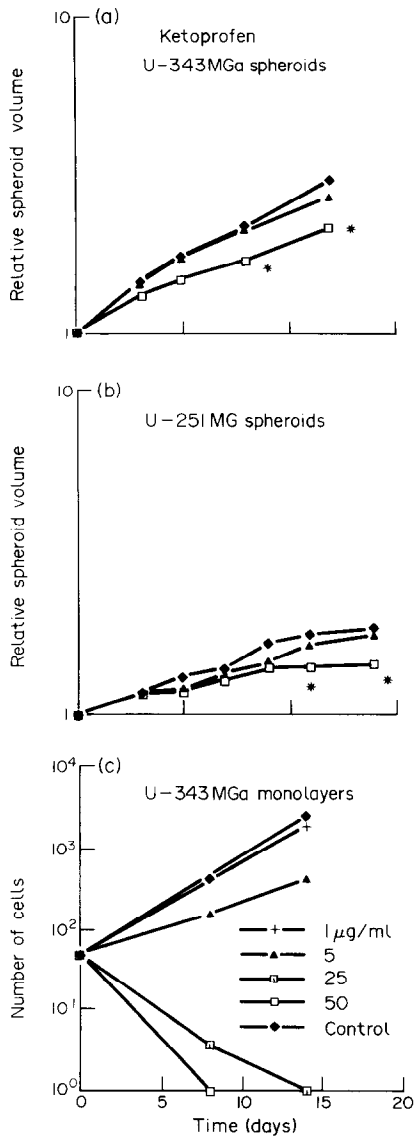

Fig. 4. Growth curves for monolayers and spheroids after ketoprofen treatment. S.D.s were less than 30% of mean values in each point. (No. of spheroids of the significantly different graphs: 8 control, 9 treated and 5 control, 8 treated in (a) and (b), respectively.)

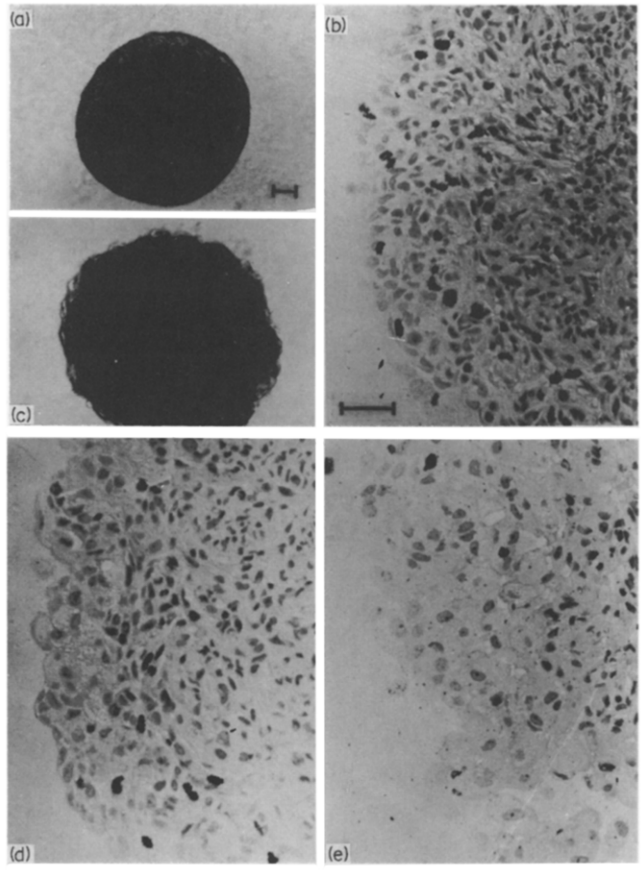

Fig. 5. NDGA (hydroquinone-form) treated spheroids became larger with rough surfaces (c) compared with controls (a). Histological investigation and autoradiography showed cell swelling, loosened cell to cell contacts, and a considerable decrease in labelling index. U-343 MGa spheroids were examined in overlay culture (a, c). ^{3}H-thymidine-labelled haematoxylin-stained U-343MGa: control (b), 10 (d), and 100 μM (e) spheroids. Bars = 50 μm.

28]. However, we found no dramatic effects on the growth of the spheroids. A possible explanation for this difference could be that the effects of dexamethasone are mediated through glucocorticoid receptors. The glioma cell-lines U-118MG and U-251MG do bind at least some dexamethasone [29] so they probably do not lack receptors. However many gliomas lack or have low levels of glucocorticoid receptors [30]. *In vivo* endothelial cells, macrophages, and leucocytes all have large amounts of glucocorticoid receptors and could therefore mediate a stronger effect. Thus the therapeutic effects of glucocorticoid treatment in patients might be an indirect effect.

Quinacrine had a dramatic effect on the growth of the spheroids, especially at higher concentrations. The retardation of growth was accompanied by gross morphological changes, with cells shedding from the spheroid surface. This could be a direct cytotoxic effect of quinacrine, not necessarily related only to phospolipase-A_2 inhibition [31]. The growth inhibitory effect of 0.1 μmol/l quinacrine was not accompanied by decreased

Table 1. *Labelling indices*

Drug (cell type)*	Labelling index		
Quinacrine (U-118MG)	*0.1 μmol/l*	*1.0 μmol/l*	*Control*
0–100 μm	12.5 (±2.1)	14.5 (±2.4)	11.4 (±0.8)
100–200 μm	2.7 (±0.8)	3.0 (±0.9)	3.3 (±0.5)
Indomethacin (U 343MGa)	*5.0 μg/ml*	*50.0 μg/ml*	*Control*
0–100 μm	13.4 (±2.2)	11.2 (±2.1)	15.0 (±3.1)
100–200 μm	4.1 (±0.5)	3.6 (±0.3)	3.4 (±0.4)
NDGA (U 343MGa)	*10 μmol/l* (Q)	*10 μmol/l* (Hq)	*Control*
0–100 μm	14.2 (±2.5)	6.8 (±2.1)†	15.0 (±3.1)
100–200 μm	0.8 (±0.2)†	0†	3.4 (±0.4)
Ketoprofen (U 251MG)	*5.0 μg/ml*	*50.0 μg/ml*	*Control*
0–100 μm	13.5 (±5.1)	8.9 (±2.9)†	16.4 (±4.0)
100–200 μm	7.7 (±3.8)	3.7 (±1.1)	2.7 (±1.1)

Mean (S.D.) of five spheroids diameters 650–750 μm.
*Depth = distance from spheroid surface.
†Significantly different from control ($P \leq 0.05$).
Q = quinone and Hq = hydroquinone form.

^{3}H-thymidine labelling. So this effect could be the result of structural changes of spheroids, which might relate to the inhibition of arachidonic acid release.

Indomethacin is a potent prostaglandin inhibitor, with slight stimulation of leukotriene production at intermediate dose levels. NDGA is a leukotriene inhibitor with no known effects on prostaglandin production. At lower concentrations indomethacin had a stimulatory effect, whereas NDGA had an inhibitory effect on spheroid volume growth. The labelling index showed no great differences after indomethacin, except for a slight decrease in the outer portion of spheroids. NDGA significantly decreased the proliferating labelled cells in the inner region with the quinone form, and in the whole spheroids after treatment with hydroquinone form, a strong lipoxygenase inhibitor.

The pattern of ketoprofen-induced disturbances followed that of indomethacin except for the lack of stimulation of growth at low concentrations. The inhibitory effects on volume growth and cell proliferation at 50 μg/ml were significant.

It is possible that inhibition of prostaglandins increased the permeability of the cells, resulting in swelling. The accompanying increase in permeability in the whole spheroid might generate more favourable conditions for the deeper cell layers. An increase in permeability might relate to inhibition of prostaglandins but could also relate to an imbalance in favour of leukotriene production, which is known to increase membrane permeability [15].

We found a significant decrease in cell proliferation in the peripheral as well as in the deeper region after strong lipoxygenase inhibition (hydroquinone-NDGA). Besides the inhibitory effect on cell respiration of this drug [24], the effect on proliferation could be explained by the inhibitory effect on leukotriene production [32]. The lack of inhibitory effect on spheroid growth could be explained by combined morphological changes.

It seems as if prostaglandin inhibition (indomethacin) also has a small direct antiproliferative effect, as seen by the decrease in the labelling index of the peripheral cells. This effect could be related to lack of some prostaglandins which stimulate proliferation. The stronger growth inhibitory effect of ketoprofen compared with indomethacin could be explained by the dual cyclo-oxygenase and lipoxygenase inhibitory potency of this drug.

In the monolayer cultures the patterns of growth changes were more pronounced than in the spheroid cultures, with a stimulatory effect by indomethacin and a clear growth inhibition of NDGA and ketoprofen.

Our experiments are not sufficient to explain the mechanisms of action of the inhibitors. The interactions are too complex to allow simple conclusions. The effects were statistically significant in some cases although the induced changes were not large. It is, however, clear that growth of the human glioma spheroids was affected by inhibitors of the arachidonic acid cascade. The effects on the monolayer cultures were somewhat stronger than on the corresponding spheroid cultures. It is, however, necessary to consider the influences seen in spheriod growth not only as indicative of proliferative or anti-proliferative effects, but also of other phenomena such as swelling of cells, changes in the volume of extracellular matrix, and volume changes of the central necrosis. Furthermore, the growth patterns of the spheroids might be affected by factors such as penetration into and out of the spheroids, which might secondarily change proliferation. As our results demonstrated, the structure of spheroids could be affected by these drugs, effects that in volume changes might have clinical significance. Therefore detailed morphological studies on spheroids exposed to more selective inhibitors of arachidonic acid cascade would be worthwhile.

The effects on the growth patterns of the spheroids must result from the sum of effects of different prostaglandins and different leukotrienes, each with their own action. To evaluate the role of arachidonic acid metabolites it will be necessary to study each separate biochemical pathway in a more specific way. Selective compounds to allow this are, for most steps, unfortunately not available. A few selective inhibitors, analogues, and antagonists have been introduced which could be used for further studies.

1. Ceuppens J, Goodwin J. Prostaglandins and the immune response to cancer (review). *Anticancer Res* 1981, **1**, 71–78.
2. Goodwin JS. Prostaglandins and host defense in cancer. *Med Clin North Am* 1981, **65**, 829–841.
3. Drago JR, Rohner TJ, Demers LM. The synthesis of prostaglandins by the Nb rat prostate tumor. *Anticancer Res* 1985, **5**, 393–396.
4. Lauro GM, Di Lorenzo N, Grossi M, Maleci A, Guidetti B. Prostaglandin E2 as an immunomodulating factor released *in vitro* by human glioma cells. *Acta Neuropathol* 1986, **69**, 278–282.
5. Cooper C, Jones HG, Weller RO, Walker V. Production of prostaglandins and thromboxane by isolated cells from intracranial tumours. *J Neurol Neurosurg Pysch* 1984, **47**, 579–584.
6. Kort WJ, Weijma IM, Bijma AM, van Schalkwijk WP, Zijlstra FJ, Westbroek DL. Growth of an implanted fibrosarcoma in rats is associated with high levels of plasma prostaglandin-E2 and thromboxane-B2. *Prostaglandins Leukotrienes Med* 1987, **28**, 25–34.
7. Farrell CL, Megyesi J, Del Maestro RF. Effect of ibuprofen on tumor growth in the C6 spheroid implantation glioma model. *J Neurosurg* 1988, **68**, 925–930.
8. Dawson W, Boot JR, Harvey J, Walker JR. The pharmacology of benoxaprofen with particular reference to effects on lipoxygenase product formation. *Eur J Rheumatol Inflamm* 1982, **5**, 61–68.
9. Karmali R, Marsh J. Antitumor activity in rat mammary adenocarcinoma: the effect of cyclooxygenase inhibitors and immunization against prostaglandin E_2. *Prostaglandins Leukotrienes Med* 1986, **23**, 11–14.
10. Lynch NR, Castes M, Astoin M, Salamon J-C. Mechanisms of inhibition of tumor growth by aspirin and indomethacin. *Br J Cancer* 1978, **38**, 503–512.
11. Lynch NR, Salomon J-C. Tumor growth inhibition and potentiation of immunotherapy by indomethacin in mice. *J Natl Cancer Inst* 1979, **62**, 117–121.
12. Rubio CA. Further studies on the therapeutic effect of indomethacin on esophageal tumors. *Cancer* 1986, **58**, 1029–1031.
13. Meerpohl HG, Bauknecht T. Role of prostaglandins on the regulation of macrophage proliferation and cytotoxic functions. *Prostaglandins* 1986, **31**, 961–972.
14. Parker CW. Lekotrienes and prostaglandins in the immune system. In: Zor V *et al.* eds. *Advances in Prostaglandin, Thromboxane and Leukotriene Research*. New York, Raven Press, 1986, Vol 16, 113–134.
15. Ford-Hutchinson AW. Leukotrienes: Biological properties, evidence for specific receptor sites and evidence for the involvement of leukotrienes in pathological situations. In: Lands WEM ed. *Biochemistry of Arachidonic Acid Metabolism*. Boston, Martinus Nijhoff, 1985, 269–285.
16. Nederman T, Norling B, Glimelius B, Carlsson J, Brunk U. Demonstration of an extracellular matrix in multicellular tumor spheroids. *Cancer Res* 1984, **44**, 3090–3097.
17. Sutherland RM. Cell and environment interactions in tumor microregions: the multicell spheroid model. *Science* 1988, **240**, 117–256.
18. Acker H, Carlsson J, Holtermann G, Nederman T, Nylén T. Influence of glucose and buffer capacity in the culture medium on growth and pH in spheroids of human thyroid carcinoma and human glioma origin. *Cancer Res* 1987, **47**, 3504–3508.
19. Carlsson J, Acker H. Relations between pH, oxygen partial pressure and growth in cultured cell spheroids. *Int J Cancer* (in press).
20. Freyer JP. Role of necrosis in regulating the growth saturation of multicellular spheroids. *Cancer Res* 1988, **48**, 2432–2439.

21. Westermark B. The deficient density dependent growth control of human malignant glioma cells and virus transformed glia-like cells in culture. *Int J Cancer* 1973, **12**, 438–451.
22. Carlsson J, Yuhas JM. Liquid overlay culture of cellular spheroids. In: Acker H, Carlsson J, Durand RE, Sutherland RM eds. *Spheroids in Cancer Research: Methods and Perspectives*. Berlin, Springer 1984, 1–23.
23. Blackwell GJ, Flower RJ. Inhibition of phospholipase. *Br Med Bull* 1983, **39**, 260–264.
24. Burk D, Woods M. Hydrogen peroxide, catalase, glutatione peroxidase, quinons, nordihydroguaiaretic acid, and phosphopyridine nucleotides in relation to X-ray action on cancer cells. *Radiation Res Suppl* 1963, **3**, 212–246.
25. Carlsson J, Nilsson K, Westermark B, Pontén J, Sundström C, Larsson E, Bergh J, Pålmann S, Busch L, Collins VP. Formation and growth of multicellular spheroids of human origin. *Int J Cancer* 1983, **31**, 523–533.
26. Carlsson J. A proliferation gradient in three dimensional colonies of cultured human glioma cells. *Int J Cancer* 1977, **20**, 129–136.
27. Hatam A, Yu Z-Y, Bergström M, Berggren B-M, Greitz T. Effect of dexamethasone treatment on peritumoral brain edema: evaluation by computed tomography. *J Comput Assist Tomogr* 1982, **6**, 586–592.
28. Hatam A, Bergström M, Yu Z-Y, Granholm L, Berggren B-M. Effect of dexamethasone treatment on volume and contrast enhancement of intracranial neoplasms. *J Comput Assist Tomogr* 1983, **7**, 295–300.
29. Millar BC, Jinks S. Studies on the relationship between the radiation resistance and glutathione content of human and rodent cells after treatment with dexamethasone *in vitro*. *Int J Radiation Biol* 1985, **47**, 539–552.
30. Rengachary SS, Tilzer LL. A study of dexamethasone receptor protein in human gliomas. *J Surg Research* 1981, **31**, 447–455.
31. Hirata F, Toyoshima S, Axelrod J, Waxdal J. Phospholipid methylation: a biochemical signal modulating lymphocyte mitogenesis. *Proc Natl Acad Sci USA* 1980, **77**, 862.
32. Paratheofanis FJ, Lands WEM. Lipoxygenase mechanisms. In: Lands WEM ed. *Biochemistry of Arachidonic Acid Metabolism*. Boston, Martinus Nijhoff, 1985, 9–39.

Acknowledgements—This work was supported by grants from the Swedish Cancer Society (1176-B89-02XB, 89:175 and 2192-B88-02X) and the Swedish Medical Research Council (B87-39X-07004-03B).

Eur J Cancer, Vol. 26, No. 7, pp. 807–810, 1990.
Printed in Great Britain

0277-5379/90$3.00 + 0.00
Pergamon Press plc

Steroid Receptors in Human Osteoblast-like Cells

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The presence and functions of steroid receptors were evaluated in three human osteosarcoma cell lines (OS1 = SA OS; OS2 = HOS TE 85, and OS3 = MNNG HOS TE 85). The human breast cancer cell line MCF-7 was used as internal control for oestrogen receptors (E_2R). High and low affinity sites were characterised. The high affinity sites had a similar dissociation constant in all four cell lines. In contrast, the number of sites per cell was higher in MCF-7 cells. E_2 did not significantly modify the number of progesterone receptors (PgR) per cell in any of the osteosarcoma lines. As expected, E_2 increased the number of PgR sites per MCF-7 cell. 4-hydroxytamoxifen decreased the growth of MCF-7 cells only. OS1 and OS2 were sensitive only to the highest concentration tested, which produces only non-specific cytotoxic effects. Thus E_2R and PgR were found in osteoblast-like cells, but the function of E_2R in such cells remains unknown.
Eur J Cancer, Vol. 26, No. 7, pp. 807—810, 1990.

INTRODUCTION

ALTHOUGH BOTH estradiol (E_2) and progesterone (Pg) are involved in the regulation of bone metabolism [1], bone cells were generally not thought to contain steroid receptors [2, 3]. In 1988, Kaplan *et al.* [4] described E_2 receptors (E_2R) in bone from a patient with McCune–Albright syndrome. More convincing evidence was provided by Eriksen *et al.* [5], who reported osteoblast-like cells displaying steroid-specific, saturable, and temperature-dependent nuclear binding. In breast cancer, E_2R and PgR assays predict response to endocrine treatment [6] in addition to having a prognostic value [7]. Our study was designed to evaluate the presence of E_2R and PgR in human osteosarcoma cell lines, and to study the functions of such receptors. We used three cell lines derived from human osteosarcomas. The human breast cancer cell line, MCF-7, known to be E_2R and PgR positive, was used as internal control.

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