Suppression of Human Cancer Cell Proliferation by Lipoxygenase Inhibitors and Gamma-Radiation *in vitro*

J. HOFMANOVÁ, E. MUSILOVÁ and A. KOZUBÍK

Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, 612 65 Brno, Czech Republic

Abstract. The effects of inhibitors of arachidonic acid oxidative metabolism, gamma-radiation and/or their combinations on proliferation and cell cycle were studied in human breast carcinoma HS578T and monoblastoid U937 cell lines. While piroxicam, an inhibitor of cyclooxygenase pathway, had no significant effects on cell proliferation, inhibitors of lipoxygenase pathway, nordihydroguaiaretic acid and esculetin, suppressed [^{3}H]-thymidine incorporation and cell growth. The latter agents also differed in their modulation of cell cycle parameters depending on the cell line and the time of treatment. When the cells were preirradiated with gamma radiation (5 Gy) and treated with the drugs (at concentrations 50 μmol/l and higher) the effects on cell proliferation were mostly additive. On the other hand, the results suggest that antiproliferative effects could be significantly strengthened when lower doses (25 μmol/l) of lipoxygenase inhibitors were combined with a low dose (1 Gy) of gamma-radiation. Experiments monitoring the reversibility of the effects after single or combined treatment with the agents showed that irradiation suppressed the ability of U937 cells to restore cell proliferation, and that these effects may be strenghtened by esculetin. In conclusion, our results (1) suggest that the lipoxygenase pathway plays a significant role in proliferation of cancer HS578T and U937 cells *in vitro*, and (2) implicate the possibility of more effective antiproliferative effects after combined treatment of cells with gamma-radiation and lipoxygenase inhibitors.

Key words: Arachidonic acid — Lipoxygenase — Gamma-radiation — Cell cycle — Cancer cells

Introduction

Cell stimulation is often associated with the activation of the enzyme phospholipase A_2 which mediates the release of a polyunsaturated fatty acid – arachidonic

acid (AA). Oxidative metabolism of AA generates a number of bioactive derivatives, eicosanoids: prostaglandins (PGs) and thromboxanes formed through the cyclooxygenase (CO) pathway; hydroperoxy acids and leukotrienes (LTs) formed by the action of lipoxygenases (LPOs); and other AA metabolites generated through the cytochrome P450/ monooxygenase pathway. Recently, numerous studies have demonstrated that eicosanoids are involved in both intercelullar and intracelullar signalling, and thus in the regulation of cell proliferation and differentiation (for review see DiMarzo 1995).

It has been shown that especially PGs and LTs are also involved in specific steps of cell transformation, tumour growth, and formation of metastases (Honn and Marnett 1984; Fulton 1984; Goodwin and Ceuppens 1985). Compounds which prevent AA release from the cell membrane or block some of its characteristic enzymatic pathways make it possible to determine the dependence of the tumour cell proliferation on the particular type of AA metabolites (Ara and Teicher 1996). Experimental and clinical studies have brought evidence that nonsteroidal anti-inflammatory drugs (NSAIDs), such as indomethacin, piroxicam or aspirin, which all inhibit the CO pathway, may slow down the development and reduce the growth of some tumours, in particular *in vivo* (Furuta et al. 1988; Earnest et al. 1992). Also, administration of LPO inhibitors has been shown to decrease proliferation of various human cancer cell lines *in vitro* (Snyder et al. 1989; Gáti et al. 1990; Rose and Connoly 1991; Anderson et al. 1992), as well as tumorigenesis *in vivo* (Noguchi et al. 1993).

Although the role of eicosanoids in cancer cell proliferation has been studied extensively, further precise knowledge of the role of individual AA metabolites in the proliferation and differentiation of malignant cells is essential for an effective antitumour therapy. To the best of our knowledge, there has been no study conducted to monitor the effects of LPO inhibitors on the cell cycle in this context. Moreover, current trends in cancer therapy explore the possibilities of combined use of various therapeutic modalities. Ionizing radiation seems to be a promising candidate for the combined use with inhibitors of eicosanoid metabolism, since in addition to damaging DNA, it also induces essential changes in cell membranes, and activates events associated with the metabolism of polyunsaturated fatty acids (Walden and Farzaneh 1990). This prompted us to study the effects of piroxicam (inhibitor of CO), nordihydroguaiaretic acid (inhibitor of 5-LPO and, at higher concentrations, also of CO and cytochrome P450/monooxygenase pathways), and esculetin (inhibitor of 5- and 12-LPO), on the proliferation and cell cycle characteristics of two human cancer cell lines.

Abbreviations: AA – arachidonic acid; PGs – prostaglandins; LTs – leukotrienes; LPOs – lipoxygenases; 5-LPO – 5-lipoxygenase; CO – cyclooxygenase; NSAIDs – nonsteroidal anti-inflammatory drugs; FCS – fetal calf serum; PIROX – piroxicam;

ESCUL – 6, 7-dihydroxycoumarin (esculetin); NDGA – nordihydroguaiaretic acid; [^{3}H]-dT – tritiated thymidine; ANOVA – analysis of variance;

Materials and Methods

Cell cultures

Human breast carcinoma cell line (HS578T) was maintained in Dulbecco's modified Eagle's medium supplemented with 10% bovine fetal calf serum (FCS, Sebak, Germany), 10 μg/ml bovine insulin, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were harvested by trypsinization. Human monoblastoid cell line (U937) was maintained in RPMI-1640 medium supplemented with 10% FCS and the same amount of antibiotics used for cultivation of HS578T cells. Cells were incubated at 37 °C in a humidified incubator with 5% CO_2. The media and insulin were purchased from Sigma Chemical Co. (St. Louis, MO). Cell lines were obtained from European Collection of Animal Cell Cultures (ECACC, Porton Down, Salisbury, UK).

Drug treatment

Piroxicam (PIROX), 6,7-dihydroxycoumarin (esculetin, ESCUL), and nordihydroguaiaretic acid (NDGA) (all products of Sigma) were dissolved in ethanol and added to the cultivation medium in appropriate concentrations of the order of μmol/l, 3 hours after seeding the cells. Parallel control tests were performed with corresponding concentrations of ethanol (max. 0.5%), which showed no effects on any of the parameters tested.

Cell viability

The cell viability was determined microscopically by the trypan blue (0.4%) exclusion assay as the percentage of viable (unstained) cells of the total (200) cells counted.

Irradiation

Cells were exposed to gamma rays (^{60}Co, dose rate 0.4 Gy/min) 3 hours after seeding. The control groups were sham-irradiated. The drugs were added to the cultivation medium immediately after the radiation exposure.

Evaluation of the cell proliferation

Cell growth. Exponentially growing cells were harvested, resuspended in the respective medium, and seeded in 24-well plastic dishes (Nunc) in concentrations of 1×10^5 cells/ml (U937) or 3×10^4 cells/ml (HS578T). The cell counts were determined every day using Coulter Counter (model ZF). Each experiment was performed in triplicate.

[^{3}H]-thymidine incorporation. Cells were seeded in 96-well plastic dishes (Nunc) in six parallels for each sample, and cultivated for 24 or 72 h. [^{3}H]-thymidine ([^{3}H]-dT, 3.7 kBq/ml, spec. activity 980 GBq/mmol) was added to the medium for the last two hours of cultivation. The cells were then harvested onto Whatman filter glass-fiber paper using an automatic harvester. Radioactivity was measured by liquid-scintillation counting.

Flow cytometry

Cells were seeded as indicated for the determination of cell growth. After various time intervals of cultivation the cells were harvested, cooled on ice, washed with cold phosphate-buffered saline solution, and stained with propidium iodide (10 μg/ml) in Vindelov's solution (1 mol/l Tris, NaCl, 0.1% Triton X 100, 0.7 mg/ml RNAse A) for 30 min at 37°C. Fluorescence was measured with an Epics Profile II flow cytometer using argon ion laser at 488 nm for excitation. The percentages of cells in G_0/G_1, S and G_2/M phases of the cell cycle were determined in two independent experiments with the samples from three parallels pooled. 10^4 cells were analyzed for each sample using standard software.

Reversion experiments

After 72 h of cultivation of nonirradiated or irradiated cells in the presence of NDGA or ESCUL, the cells were harvested, washed with PBS, and plated in inhibitor-free medium at the same numbers as at the beginning of the experiments. They were then cultivated for another 24 h (to determinate [^{3}H]-dT incorporation and cell cycle parameters) or 72 h (to estimate growth curves).

Statistical analysis

The data are expressed as means ± S.E.M. of 2–4 experiments performed in 3–6 parallels. The two-tailed paired t-test was used to determine statistical differences between control and experimental groups. For statistical evaluation of combined effects of radiation and drugs on [^{3}H]-dT incorporation, the analysis of variance (ANOVA) was used. The null hypothesis for the interaction between two combined factors was that the response of [^{3}H]-dT incorporation did not differ between specific levels of one factor depending upon a particular level of the second factor. All the results were presented as percentages of [^{3}H]-dT incorporation levels in control samples.

Results

We studied the effects of various inhibitors of eicosanoid biosynthesis on parameters which reflect the proliferation of HS578T and U937 cells using a wide range of concentrations (12.5–100 μmol/l). The results showed that NDGA and ESCUL

significantly decreased the cell growth and the incorporation of [^{3}H]-dT in comparison with nontreated control, in a concentration- and time-dependent manner without decreasing the cell viability (except for NDGA at concentrations exceeding 50 μmol/l which decreased the cell viability by about 20–40%). On the other hand, PIROX exhibited no or even stimulative (HS578T cells) effects on cell proliferation. Relevant data documenting these results are shown in Figs. 1 and 3 *A*, *B*.

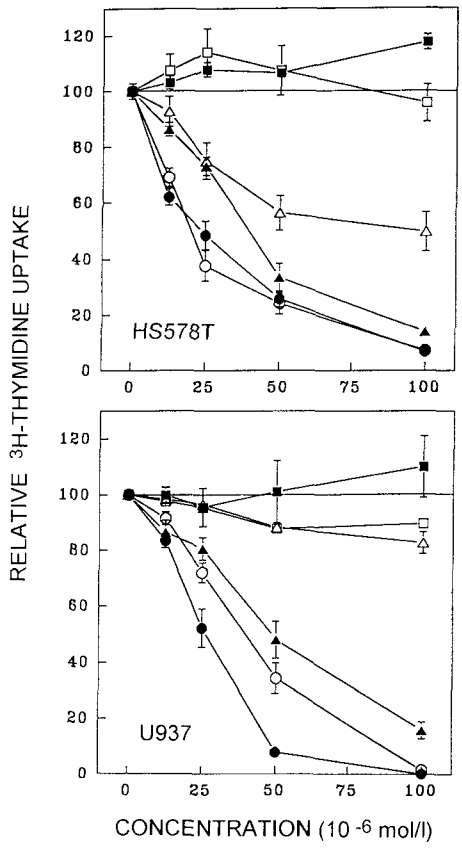

Figure 1. Effects of various doses of PIROX (squares), NDGA (circles), or ESCUL (triangles) on ^{3}H-thymidine incorporation (% of nontreated control) in HS578T and U937 cells after 24 h (open symbols) or 72 h (solid symbols) cultivation. The data are means ± S.E.M. for 3–4 independent experiments performed in six parallels.

After exposure to concentrations corresponding to approximately IC_{50}/72 h for ^{3}H-thymidine incorporation, i. e. 25 μmol/l NDGA and 50 μmol/l ESCUL, changes of the cell cycle parameters were studied during 72 h of cultivation using flow cytometry (Table 1, first column). Control populations of HS578T and U937 had similar patterns of cell cycle distribution, i. e. about 58% cells in G_0/G_1; 12% in S; and 28% in G_2/M phase. NDGA increased the counts of U937 cells in either G_2/M phase (after 24 h up to 39%) or G_0/G_1 (after 48 and 72h up to 65%),

Table 1. Cell cycle distribution of nontreated control (C) U937 and HT578T cells and cells upon treatment with nordihydroguaiaretic acid (NDGA, 25 μmol/l), esculetin (ESCUL, 50 μmol/l) and/or gamma-radiation (5 Gy)

U937 cells

Hours	Treatment	Nonirradiated			Irradiated		
		G_0/G_1	S	G_2/M	G_0/G_1	S	G_2/M
0	C	59	13	28		–	
3	C	64	11	25	55	12	32
	NDGA	60	13	26	59	13	27
	ESCUL	58	15	27	56	15	29
6	C	57	13	30	46	16	37
	NDGA	52	18	28	56	13	30
	ESCUL	47	17	36	48	13	38
24	C	56	14	29	38	7	58
	NDGA	17	13	39	38	11	51
	ESCUL	17	21	31	37	9	56
48	C	59	12	28	49	9	37
	NDGA	65	3	30	55	7	34
	ESCUL	62	13	24	49	10	38
72	C	62	12	27	51	9	32
	NDGA	64	8	27	54	7	31
	ESCUL	55	10	33	48	9	30
72 +24 R[a)]	C	57	11	31	54	12	27
	NDGA	61	10	27	56	7	29
	ESCUL	69	5	23	46	6	38

[a)] cells were cultivated with the drugs for 72 h and subsequently in drug free medium for additional 24 h

and simultaneously reduced cell numbers in S phase as compared with nontreated control cells. ESCUL increased the number of cells in S phase of the cell cycle (during 24 h up to 21%). This effect was more pronounced at higher concentration of ESCUL (100 μmol/l) (up to 35% of cells in S phase, data not shown). In the HS578T cell line, ESCUL induced similar changes in cell numbers in S phase (an increase of up to 19%), and NDGA increased the cell numbers in G_0/G_1 phase (after 24 h up to 75%). While during prolonged cultivation up to 72 h the values of NDGA-treated HS578T cells returned to control levels, an increase of cell number

Table 1. continued

HS578T cells

Hours	Treatment	Nonirradiated			Irradiated		
		G_0/G_1	S	G_2/M	G_0/G_1	S	G_2/M
0	C	57	12	30	–	–	–
3	C	60	8	28	55	10	37
	NDGA	62	8	28	55	10	32
	ESCUL	61	8	29	50	9	37
6	C	58	9	30	44	8	39
	NDGA	61	10	26	45	8	36
	ESCUL	65	5	28	43	6	40
24	C	58	10	29	47	12	31
	NDGA	75	6	16	57	7	27
	ESCUL	58	19	20	55	11	30
72	C	59	11	29	62	12	24
	NDGA	62	11	26	62	8	29
	ESCUL	33	17	48	35	11	47

in G_2/M phase (up to 48%) was observed after treatment with ESCUL. Fig. 2 shows representative results of flow cytometric analysis illustrating the cell cycle distribution 24 h after the treatment with the various agents.

Because of their antiproliferative effects, NDGA and ESCUL were used in studies of combined action with gamma-radiation on cell proliferation and cell cycle. On the basis of preliminary experiments to assess the effects of various radiation doses (1–7 Gy) on cell proliferation (data not shown), doses of 1 and 5 Gy were chosen for more detailed analyses. The dose of 1 Gy affected neither proliferation nor cell viability, and 5 Gy reduced cell proliferation by 20–40% without influencing cell viability (as compared with nonirradiated control) during the time period tested. After irradiation with the above doses, the cells were cultivated in media at various concentrations of NDGA or ESCUL, and their growth, viability, [^{3}H]-dT incorporation, and the cell cycle were compared with reference data obtained after the exposure to single factors. The growth of HS578T and U937 cells was significantly reduced (at least $p < 0.01$) after the irradiation (5 Gy), after the treatment with 25 μmol/l NDGA, 50 μmol/l ESCUL or after combined exposure to radiation and either of the inhibitors, as compared with nontreated control (Fig. 3 *A*, *B*).

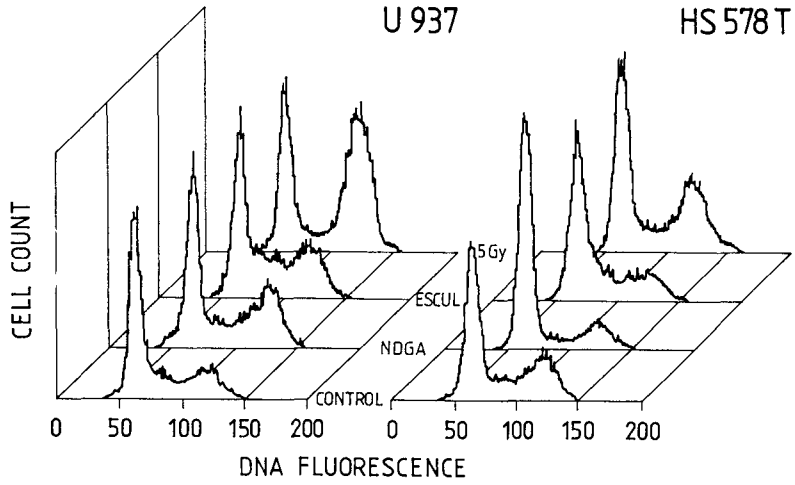

Figure 2. Representative results of flow cytometric analysis of the cell cycles of U937 and HS578T cell lines after 24 h of cultivation. Cells were treated with 25 μmol/l NDGA, 50 μmol/l ESCUL, or irradiated with 5 Gy.

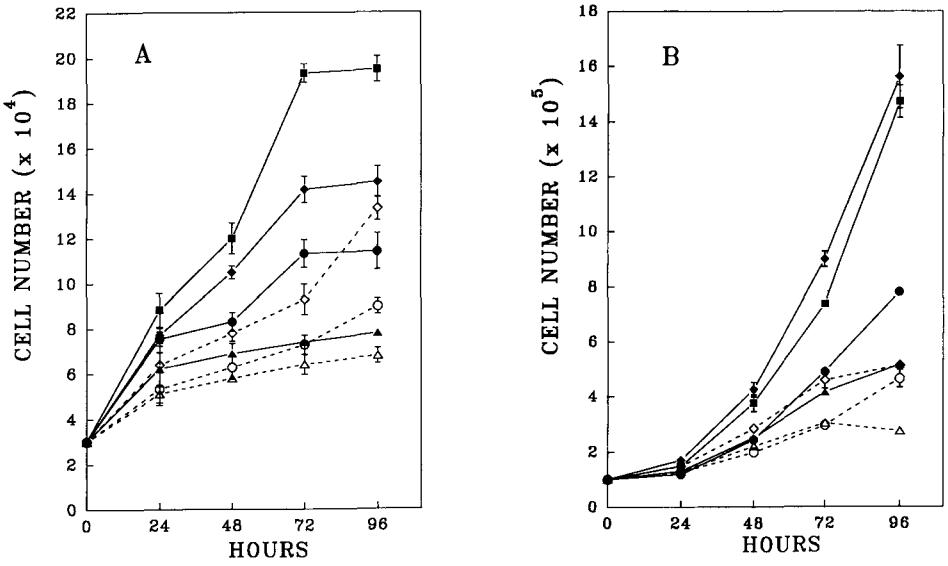

Figure 3. Growth of HS578T (*A*) and U937 (*B*) cells cultivated in the absence (diamonds) or in the presence of 50 μmol/l PIROX (squares), 25 μmol/l NDGA (circles) or 50 μmol/l ESCUL (triangles). The solid lines and solid symbols represent nonirradiated cells. The dashed lines and open symbols represent cells irradiated with 5 Gy. The data are means ± S.E.M. for three independent experiments performed in triplicates.

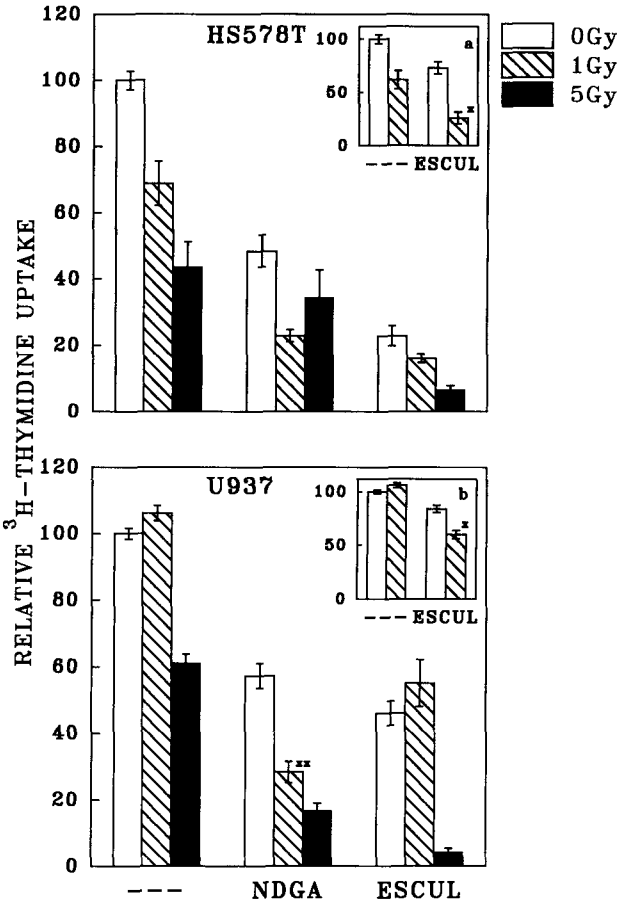

Figure 4. ^{3}H-thymidine incorporation (% of nontreated control) in HS578T and U937 cells subjected to different treatment. The nonirradiated cells or cells irradiated with 1 or 5 Gy were cultivated in the presence of 25 μmol/l NDGA, 25 μmol/l ESCUL (inserts 3 *a*, *b*) or 50 μmol/l ESCUL or without the agents (– – –) for 72 h. Data are means ± S.E.M. for three independent experiments performed in six parallels. * $p < 0.05$; ** $p < 0.01$ significance of the interactive component

During the same time interval, the cell viability was slighly decreased upon combined action of 25 μmol/l NDGA and radiation (about 80%) only. Incorporation of [^{3}H]-dT into the cells after treatment with LPO inhibitors and irradiation (1 or 5 Gy) was studied in further experiments. After 72 h of cultivation, irradiation (with the exception of the dose of 1 Gy in U937 cells), NDGA, ESCUL alone, and their combination with irradiation significantly ($p < 0.01$ or more) reduced the in-

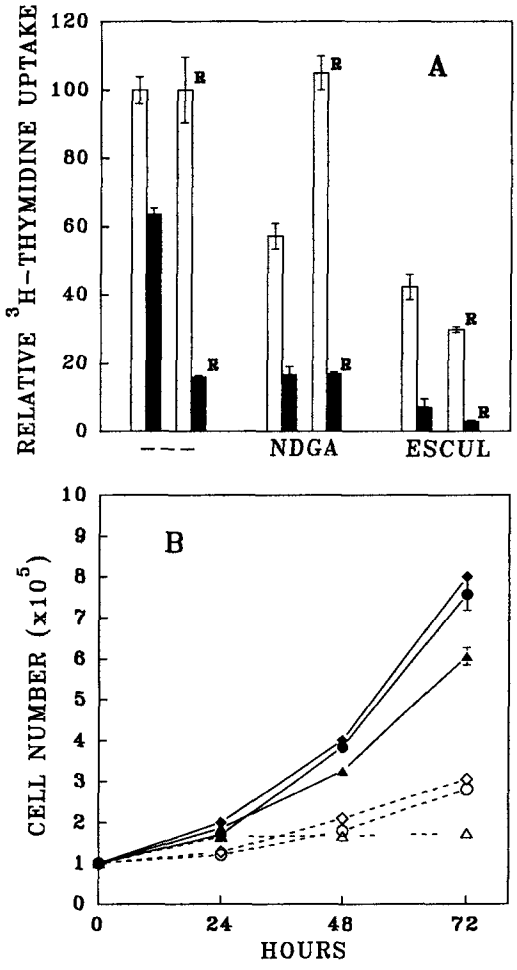

Figure 5. Reversion experiments incorporation of ^{3}H thymidine (*A*) and growth (*B*) in U937 cells *A* ^{3}H-thymidine incorporation (% of nontreated control) Nonirradiated cells or cells irradiated with 5 Gy were first cultivated in the absence ( ) or in the presence of 25 μmol/l NDGA or 50 μmol/l ESCUL for 72 h (bars without symbol R) and then cultivated for 24 h in drug free medium (bars with symbol R) Open bars nonirradiated solid bars irradiated with 5 Gy Each value represents means ± S E M of two independent experiments performed in six parallels *B* Cell growth in drug free medium after pre-treatment with 25 μmol/l NDGA (circles) or 50 μmol/l ESCUL (triangles) Diamonds represent nontreated controls The solid lines and solid symbols represent nonirradiated cells the dashed lines and open symbols represent cells irradiated with 5 Gy The data are means ± S E M for two independent experiments performed in triplicates

corporation of [^{3}H]-dT into both cell types as compared with nontreated controls More detailed statistical analysis (ANOVA) revealed that combined effects of the

drugs and irradiation were rather of additive nature. Significant interactive effects on [^{3}H]-dT incorporation were demonstrated for the combinations of 1 Gy with 25 μmol/l NDGA or 25 μmol/l ESCUL (Fig. 4).

Flow cytometric analysis of cell cycle showed that irradiation with 5 Gy caused an increase in the counts of U937 cells in G_2/M phase (up to 58%) after 24 h of cultivation (Fig. 2., Table 1, second column). The values returned to those of nonirradiated control thereafter. In the HS578T cell line, the increase in cell counts in G_2/M phase was not so marked. After combined action of irradiation (5 Gy) and 25 μmol/l NDGA or 50 μmol/l ESCUL, the cell cycle parameters were similar to those observed after the treatment with the individual factors alone (Table 1).

The reversibility of the effects of the single factors and of their combination with irradiation (5 Gy) in U937 cells is shown in Fig. 5. The results showed that after 72 h of irradiation with 5 Gy or treatment with 25 μmol/l NDGA or 50 μmol/l ESCUL, the incorporation of [^{3}H]-dT into the cells was significantly decreased (at least $p < 0.01$) as compared with nontreated controls, and that after subsequent 24 h-cultivation of the cells in a drug-free medium, the values continued to decrease, with the exception of the cells treated with NDGA alone. The suppressive effect of the combination of irradiation and ESCUL (3.5% of control value) was the most pronounced (Fig. 5 *A*). The effects on [^{3}H]-dT incorporation are in good agreement with those on cell growth, the latter returning to control values after the termination of the NDGA treatment, remaining reduced after the action of ESCUL alone, irradiation alone, or after the combination of irradiation and NDGA, and coming to a complete halt after the combined exposure to ESCUL and irradiation (Fig. 5 *B*). The combination of irradiation with ESCUL also prevented the reversibility of the effects on the cell cycle, i. e. it caused a stable increase of the cell counts (38%) in G_2/M phase in comparison with the effects of ESCUL or irradiation alone (23 and 27%, respectively; Table 1).

Discussion

Our results showed that while piroxicam, a CO inhibitor, had no or even stimulative effect on the proliferation of leukemia (U937) or breast cancer (HS578T) cells, both LPO-inhibiting agents, NDGA and esculetin, decreased proliferation parameters and modulated the cell cycle without significant changes in cell viability. Although the CO-inhibiting NSAIDs are reported to be very potent in chemoprevention of bladder and colon cancers in animal models and in man (Earnest et al. 1992; Reddy et al. 1992), conclusions from the results obtained in other experimental systems have been inconsistent (Fulton 1984; Fukushima 1990; Beckerman et al. 1992), probably due to different ability of individual cell types to produce PGs. On the other hand, the results observed after exposure to LPO inhibitors do not seem contradictory. Antitumour effects of 5-LPO-inhibiting substances have been

demonstrated *in vivo* (Noguchi et al. 1993) as well as in various systems *in vitro* (Snyder et al. 1989; Gáti et al. 1990; Rose and Connoly 1991; Anderson et al. 1992). This suggests a positive role of 5-LPO metabolites in the control of cancer cell proliferation including leukemic cells. These conclusions are corroborated by results which proved that the 5-LPO metabolites participate in the regulation of normal hematopoiesis both *in vitro* (Miller et al. 1986) and *in vivo* (Kozubík et al. 1993, 1994). It has been demonstrated that 5-LPO metabolites play a role in cell response to hemopoietic growth factors (Miller et al. 1993) and act directly as second messengers in the signal transduction mechanisms for some positively acting cytokines (Masongarcia et al. 1992; Peppelenbosch et al. 1992). Moreover, because growth factors play an important role also in the regulation of apoptotic cell death, the interruption of 5-LPO-dependent cell signalling can upregulate the rate of apoptosis (Tang et al. 1996). Thus, it may be speculated that the inhibition of the LPO pathway could prevent the stimulative action of growth factors on cancer cell proliferation as well as promote apoptosis.

The effects of NDGA, ESCUL, and/or irradiation on the cell cycle depended on the agent, duration of the treatment, and the cell type. To summarize, the main effects of NDGA included increased cell numbers in G_0/G_1 and decreased cell numbers in S phase of the cell cycle, while ESCUL increased the number of cells in S and/or G_2/M phases. The effect of irradiation (accumulation of cells in G_2/M phase) was stronger on the U937 cell line. The differences between the cell lines used might be explained by different growth rates, types of cultivation (U937 cells grow in suspension, HS578T cells are adherent), and possible differences in AA metabolism. Generally, the results of flow cytometry can contribute to the clarification of the mechanisms of action of individual factors and their combinations. Thus, the higher proportions of cells in S-phase 24 h after treatment by ESCUL may reflect a slower passage and accumulation of cells in this phase, which is then followed by an increase of cell numbers in G_2/M phase after 72 h of cultivation.

If the cells in our experiments were preirradiated with a dose of 5 Gy, which alone had a reducing effect on cell proliferation, and then treated with the drugs, the antiproliferative effects were enhanced, mostly in an additive manner, by the combination with either of the two drugs. However, these combinations (especially irradiation with ESCUL) can prevent reversion of inhibitory effects induced by the individual factors. It may be important from the therapeutical point of view that a low radiation dose (1 Gy), which alone does significantly not affect cell proliferation, sensitized the cells to lower doses of NDGA or ESCUL. We may conclude that specific intervention in AA oxidative pathways (namely the 5-LPO one) after irradiation interacts with, or modulates the effects of gamma-radiation, e. g. in preventing the recovery of radiation damage. These conclusions are supported by results of our other experiments not shown here. When the cells were irradiated after pretreatment with NDGA or ESCUL, no additional changes in [^{3}H]-dT

incorporation were detected

The mechanisms of combined treatment of cells with ionizing radiation and inhibitors of eicosanoid biosynthesis remain unclear Based on the facts described above it may be hypothesized that changes in the cell membrane after radiation exposure together with subsequent interruption of the 5-LPO pathway and thus probably of growth factor signalling, could result in a stronger inhibition of cell proliferation It must be stressed however that the inhibition of LPO need not be the only cause of the effects of the drugs used in our experiments Possible competitive stimulation of PG production interaction with cytochrome P 450/monooxygenase pathway (Neichi et al 1983 Wang et al 1991) or other mechanisms independent of the AA cascade might also be of importance (Huang et al 1993) Apart from these uncertainties it is evident that AA metabolic pathway especially the 5-LPO one may be a promising target for pharmacological interference with cell proliferation Our experiments show that the use of ESCUL seems more advantageous As compared with NDGA ESCUL does not affect cell viability even in concentrations exceeding 100 μmol/l and has a more pronounced effect on cell proliferation and cell cycle parameters also in combination with irradiation Although probably these effects are not selective for cancer cells LPO inhibitors in particular might represent a new pharmacological tool if combined with other known anticancer agents by reducing the effective doses and/or preventing reversion of their effects

Acknowledgements. This research is supported by grants from Grant Agency of the Czech Republic (No 505/93/2369 and No 521/96/1716)

References

Anderson K M Ondrey F Harris J F (1992) EIYA a pleotropic membrane active arachidonic acid analogues affects multiple signal transduction pathways in cultured transformed mammalian cells Clin Biochem **25,** 1 9

Ara G Teicher B A (1996) Cyclooxygenase and lipoxygenase inhibitors in cancer therapy Prostagland Leuk Essent Fatty A **54,** 3 16

Beckerman K P Schreiber R D Needleman P (1992) Cytokine modulation of immune activation associated suppression of macrophage cyclooxygenase activity in vivo Prostagland Leuk Essent Fatty A **47,** 231 238

Di Marzo V (1995) Arachidonic acid and eicosanoids as targets and effectors in second messenger interaction Prostagland Leuk Essent Fatty A **53,** 239 251

Earnest D L Hixson L J Alberts D S (1992) Piroxicam and other cyclooxygenase inhibitors potential for cancer chemoprevention J Cell Biochem Suppl **161,** 156 166

Fukushima M (1990) Prostaglandin J_2 anti-tumour and anti-viral activities and the mechanisms involved Eicosanoids **3,** 189 199

Fulton A M (1984) In vivo effects of indomethacin on the growth of murine mammary tumors Cancer Res **44,** 2416 2420

Furuta Y., Hunter N., Barkley T. Jr., Hall E., Milas L. (1988): Increase in radioresponse of murine tumors by treatment with indomethacin. Cancer Res. **48,** 3008–3013

Gáti I., Bergström M., Csóka K., Muhr C., Carlsson J. (1990): Effects of the 5-lipoxygenase inhibitors AA-863 and U-60,257 on human glioma cell lines. Prostagland. Leuk. Essent. Fatty A. **40,** 117–123

Goodwin J. S., Ceuppens J. L. (1985): Prostaglandins, cellular imunity and cancer. In: Prostaglandins and Immunity (Ed. J. S. Goodwin) pp. 1–34, M. Nijhoff Publ. Boston

Honn K. V., Marnett L. J. (1984): Prostaglandin, thromboxane, and leukotriene biosynthesis: target for antitumor and antimetastatic agents. In: Novel Approaches to Cancer Chemotherapy (Ed. P. S. Sunkara) pp. 128 163, Academic Press, Orlando

Huang H. CH., Lai M. W., Wang H. R., Chung Y. L., Hsieh L. M., Chen CH. CH. (1993): Antiproliferative effect of esculetin on vascular smooth muscle cells: possible roles of signal transduction pathways. Eur. J. Pharmacol. **237,** 39 44

Kozubík A., Hofmanová J., Holá J., Netíková J. (1993): The effect of nordihydroguaiaretic acid, an inhibitor of prostaglandin and leukotriene biosynthesis, on hematopoiesis of gamma-irradiated mice. Exp. Hematol. **21,** 138 142

Kozubík A., Hofmanová J., Pospíšil M., Netíková J., Holá J., Lojek A. (1994): Effects of drugs inhibiting prostaglandin or leukotriene biosynthesis on postirradiation haematopoiesis in mouse. Int. J. Radiat. Biol. **65,** 369–377

Masongarcia M., Clejan S., Tou J. S., Beckman B. S. (1992): Signal transduction the erythropoietin receptor - evidence for the activation of phospholipases A_2 and C. Amer. J. Physiol. **262,** C1197 C1203

Miller A. M., Weiner R. S., Ziboh V. A. (1986): Evidence for the role of leukotrienes C_4 and D_4 as essential intermediates in CSF-stimulated human myeloid colony formation. Exp. Hematol. **14,** 760 765

Miller A. M., Sandler E., Kobb S. M., Eastgate J., Zucalli J. (1993): Hematopoietic growth factor induction of gamma-glutamyl transferase in the KG-I myeloid cell line. Exp. Hematol. **21,** 9–15

Neichi T., Koshihara Y., Murota S. I. (1983): Inhibitory effect of esculetin on 5-lipoxygenase and leukotriene biosynthesis. Biochim. Biophys. Acta **753,** 130–132

Noguchi M., Kitagawa H., Miyazaki I., Mizumaki Y. (1993): Influence of esculetin on incidence, proliferation, and cell kinetics of mammary carcinomas induced by 7,12 - dimethyl - benz[a]anthracene in rats on high- and low-fat diets. Jpn. J. Cancer Res. **84,** 1010 1014

Peppelenbosch M. P., Tertoolen L. G. J., Denhertog J., Delaat S. W. (1992): Epidermal growth factor activates calcium channels by phospholipase $A_2/5$ – lipoxygenase – mediated leukotriene C_4 production. Cell **69,** 295–303

Reddy B. S., Tokumo K., Kulkarni N., Aligia C., Kelhoff G. (1992): Inhibition of colon carcinogenesis by prostaglandin synthesis inhibitors and related compounds. Carcinogenesis **13,** 1019–1023

Rose D. P., Connoly J. M. (1991): Effects of fatty acids and eicosanoid synthesis inhibitors on the growth of two human prostate cancer cell lines. Prostate **18,** 243–254

Snyder D. S., Castro R., Desforges J. F. (1989): Antiproliferative effects of lipoxygenase inhibitors on malignant human hematopoietic cell lines. Exp. Hematol. **17,** 6–9

Tang D. G., Chen Y. Q., Honn K. V. (1996): Arachidonate lipoxygenases as essential regulators of cell survival and apoptosis. Proc. Natl. Acad. Sci. USA **93,** 5241–5246

Walden T. L. Jr., Farzaneh N. K. (1990): Biological mediators. In: Biochemistry of Ionizing Radiation (Eds. T. L. Walden, Jr. and N. K. Farzaneh) pp. 55–88, Raven Press Ltd. New York

Wang Z. Y., Agarwal R., Zhou Z. C., Bickers D. R., Mukhtar H. (1991): Antimutagenic and antitumorigenic activities of nordihydroguaiaretic acid. Mutat. Res. **261,** 153–162

Final version accepted August 9, 1996