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Lipoxygenase Pathway of Arachidonic Acid Metabolism in Growth Control of Tumor Cells of Different Type

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Abstract—The influence of inhibitors of different lipoxygenases (LOX) on the growth of human tumor cells with different profiles of synthesized eicosanoids was studied. The studied LOX inhibitors had virtually no influence on the growth of A549 cells actively synthesizing cyclooxygenase and lipoxygenase metabolites of arachidonic acid (AA). The inhibitor of 12-LOX, baicalein, significantly inhibited proliferation in cultures of A431 epidermoid carcinoma cells with a characteristic domination of the major lipoxygenase metabolite of AA, 12-hydroxyeicosatetraenoic acid (12-HETE), in the profile of synthesized eicosanoids and reduced to 70% the incorporation of [^{3}H]thymidine into DNA. Treatment of these cultures with 12-HETE virtually restored the growth potential of the tumor cells. The findings suggest that the lipoxygenase metabolite of AA, 12-HETE, is a growth-limiting factor for tumor cells of definite type.

Key words: arachidonic acid metabolism, 12-HETE, human tumor cells, cell proliferation

Eicosanoids of the arachidonic acid (AA) cascade are involved in all crucial events of carcinogenesis [1-3]. In particular, cyclooxygenase AA metabolites, or prostanoids, can induce differentiation [4] and also suppress the growth of tumor cells manifesting an antiproliferative [5] or cytotoxic effect [6]. It has also been shown that the *in vitro* and *in vivo* growth of various tumor cells is limited by the major cyclooxygenase AA metabolite prostaglandin E_2 (PGE_2) synthesized in them, whereas inhibitors of cyclooxygenase in many cases display a considerable antitumor activity [7-10]. The role of main lipoxygenase AA metabolites, different monohydroxyeicosatetraenoic acids (HETE), in the control of tumor cell growth is still unclear. Recent *in vitro* studies have revealed that one of these metabolites, 15-HETE, can inhibit PGE_2-dependent proliferation and considerably suppresses synthesis of DNA in some human tumor cells [11, 12]. The ability to markedly stimulate the growth of a certain tumor cells was noted for another lipoxygenase AA metabolite, 5-HETE. 5-HETE, which is actively synthesized in MCF-7 human mammary gland carcinoma cells and PC-3 human prostate cancer cells, stimulated their proliferation depending on the dose, whereas inhibitors of 5-LOX effectively suppressed the growth of these cells [13-15]. However, the growth of human mammary gland and pancreas tumor cells was stimulated not only by 5-HETE, but also by 12-HETE [16, 17]. In this aspect, special attention should be paid to 12-HETE, which is synthesized with the involvement of different 12-LOX isoforms [18, 19] and actively modulates metastasis [20]. Expression of 12-LOX is absent in most human tumor cell lines originating from lungs, large intestine, prostate, and mammary gland [21], and at the same time 12-HETE is actively synthesized in epidermoid carcinoma cells [19].

The purpose of the present work was to study roles of different directions of the lipoxygenase pathway of AA metabolism in growth control of A549 cells (lung adenocarcinoma) and A431 cells (epidermoid carcinoma), which are fundamentally different in the ability of producing 12-HETE.

MATERIALS AND METHODS

Vessels and reagents. Plastic flasks (S = 25 cm^2) for cell cultures were obtained from Corning Costar (USA). Fetal calf serum (FCS) and the DMEM medium were purchased from Life Technologies (Great Britain); RPMI-1640 medium, inhibitors of AA metabolism NS398, indomethacin, esculetin, salicylhydroxamic acid (SHAM), and also 5-HETE, 12-HETE, and 15-HETE

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were purchased from Sigma (USA); baicalein was obtained from Fluka (Switzerland); [1-^{14}C]AA (specific activity 54 mCi/mmol) and labeled standards were obtained from Amersham (Great Britain). [Methyl-^{3}H]thymidine (specific activity 50 Ci/mmol) was prepared in the Institute of Molecular Genetics, Russian Academy of Sciences (Moscow). Other reagents of chemical purity and special purity were of domestic production.

Cell cultures. A549 human lung adenocarcinoma cells and A431 human epidermoid carcinoma cells (American Type Culture Collection, USA) were cultured at 37°C under humidified atmosphere with 5% CO_2 in a mixture of DMEM and RPMI-1640 media at the ratio 1 : 1 (total volume 5 ml) supplemented with FCS (5%), penicillin (100 U/ml), and streptomycin (100 μg/ml). The medium was usually changed every day. On the first, second, and third day of the culture growth we introduced inhibitors of AA metabolism and/or different mono-HETE. The concentration of used inhibitors was 10^{-4}-10^{-6} M, the final concentration of 5-HETE, 12-HETE, and 15-HETE was 10 ng/ml, which is close to the concentration produced endogenously and pronouncedly modulates the growth of normal and tumor cells [11, 22]. The effects of lipoxygenase metabolites of AA and inhibitors of eicosanoid biosynthesis on the cell proliferation at varied quantities of grown tumor cells were assessed on 48, 72, and 96 h of culture growth.

Assessment of cell proliferation. Proliferation of A549 and A431 cells was evaluated by the growth density of the tumor cells in culture and the intensity of [^{3}H]thymidine incorporation into DNA. [^{3}H]Thymidine (1 μCi/ml) was introduced as a 1-h pulse on the second, third, and fourth days of the culture growth, then the cells were washed thrice in the FCS-free medium to remove the excess label. For counting in a Goryaev chamber and for scintillation counting, the cells were taken off with 0.25% EDTA. The radioactivity (cpm) was determined with a Wallac 1219 liquid scintillation counter (LKB, Sweden) [23].

Study of [^{14}C]AA metabolism. Inhibitors of AA metabolism were tested *ex vivo* in cell-free homogenates of the lung tissue from DBA/2 mice. Tissue specimens of 100-200 mg to analyze eicosanoids were rapidly cut off and frozen in liquid nitrogen, then, similarly to the previously frozen centrifugates of tumor cells, were minced using an Ultra-Turrax T10 high-speed electric homogenizer (IKA-Werk, Germany) for 30 sec at 0°C in 10 volumes of 0.05 M Tris-HCl buffer (pH 7.4). After centrifugation, the supernatants (0.5 ml) were incubated with 0.5 μCi [^{14}C]AA at 37°C for 30 min. Biosynthesis of eicosanoids from [^{14}C]AA incorporated into the tumor cells was studied *in vitro* for the first-third and second-fourth days of the culture growth. The cells were incubated with the labeled precursor (0.1 μCi/ml) at 37°C for 24 h, and then the unincorporated [^{14}C]AA was removed by thrice-repeated washing with FCS-free medium. The effect of inhibitors of AA metabolism on biosynthesis of eicosanoids was assessed after 24 h incubation of labeled tumor cells with them. Eicosanoids synthesized *ex vivo* or *in vitro* were extracted twice with three volumes of ethyl acetate and afterwards the solvent was evaporated under a nitrogen flow. Metabolites of [^{14}C]AA were separated by TLC on Kieselgel 60 plates (Merck, Germany) using the organic phase of the solvent system of ethyl acetate–isooctane–CH_3COOH–H_2O (11 : 5 : 2 : 10 v/v) and labeled standards [22]. The profiles of synthesized eicosanoids on autoradiochromatograms obtained on an X-Omat XAR-5 X-ray film (Kodak, USA) were analyzed using a KS 3 densiscan (Kipp and Zonen, Holland). Radioactivity of the repeatedly extracted eicosanoids in the spots cut off from the chromatograms under study was measured with a Wallac 1219 counter. In experiments with A431 cells, for separation of the 12-HETE/15-HETE spot cut off from a TLC plate and analysis of all mono-HETE synthesized by the cells an HPLC system (Gilson, France) equipped with 305 and 302 pump models, a Linear UVIS 200 UV-detector (Linear Instruments Corp., USA), and a Zorbax C8 column (5 μm, 4.6 × 250 mm; DuPont, USA) was used. The separation was performed in a mixture of 0.1% CH_3COOH with acetonitrile at the flow rate of 1 ml/min, using a stepwise gradient of acetonitrile delivery from 30 to 100% [24], and also under an isocratic regimen with its 70% content (v/v) in the mixture.

Statistical processing of results. On determination of mean parameters of the tumor cell proliferation and biosynthesis of eicosanoids, as well as standard error of the mean and standard deviation, we used the data on three-four cultures in three independent experiments. Statistical significance of the data was evaluated with Student's *t*-test.

RESULTS

Effects of inhibitors of AA metabolism on biosynthesis of different eicosanoids in cell-free homogenates of mouse lung tissue. The selectivity of cyclooxygenase and lipoxygenase inhibitors is now an important problem. Usually the recommended inhibitors are previously tested only in monosystems with one of the enzymes, and, as a rule, their ability to suppress other metabolic pathways of AA is revealed later. The testing on cell-free homogenates of lung tissue possessing both high cyclooxygenase and lipoxygenase activities was designed to find all possible changes in the biosynthesis of eicosanoids, first of all mono-HETE, under the influence of the AA metabolism inhibitors. The most pronounced inhibitory properties were found for SHAM, which suppressed by 84 and 87% the synthesis of cyclooxygenase and lipoxygenase metabolites, respectively (Fig. 1). A selective inhibition of prostanoid production (–80% compared to the control)

was noted on the introduction of indomethacin, an equipotent inhibitor of cyclooxygenase-1 and cyclooxygenase-2 (COX-1 and COX-2). A similar selective effect on the biosynthesis of 12-HETE/15-HETE was displayed by the inhibitor of 12-LOX, baicalein (–95% compared to the control) (Fig. 1). And we also found that the inhibitor of 5-LOX, esculetin, can equally suppress the production of 5-HETE and prostanoids (–38 and –39% compared to the control, respectively), along with a slight stimulation of the synthesis of 12-HETE/15-HETE, while the specific inhibitor of COX-2, NS398, suppresses the synthesis not only of cyclooxygenase metabolites of AA (–52%) but also of 5-HETE and 12-HETE/15-HETE (–45 and –34%, respectively; Fig. 1). Thus, all studied cyclooxygenase and lipoxygenase inhibitors except indomethacin pronouncedly suppressed biosynthesis of one or another mono-HETE. Considering selectivity of the inhibitors, baicalein and esculetin seem to be especially interesting for studies on the tumor cells A549 and A431, which actively synthesize 5-HETE and 12-HETE.

Profile of metabolites synthesized from [^{14}C]AA in human epidermoid carcinoma A431 cells. We studied biosynthesis of eicosanoids by tumor cells A549 under varied culture conditions earlier [11]. Lung adenocarcinoma cells were characterized by a pronounced production of cyclooxygenase and lipoxygenase metabolites of AA with a slight predominance of 5-HETE: 5-HETE > PGE_2 > $PGF_{2\alpha}$ > 15-HETE/12-HETE.

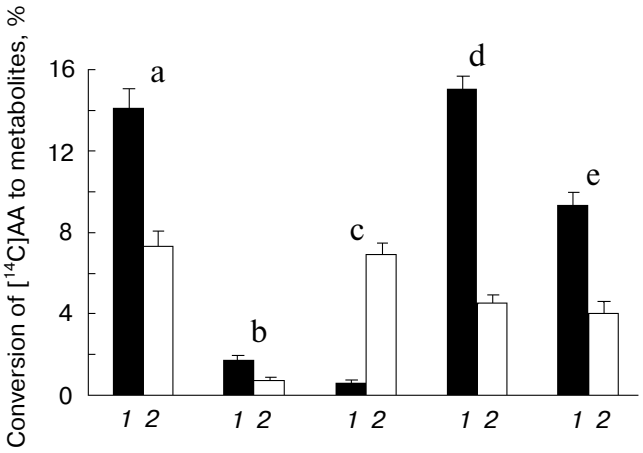

Fig. 1. Biosynthesis of 12-HETE/15-HETE (*1*) and 5-HETE (*2*) from exogenous [^{14}C]AA in control (a) homogenates of mouse lungs and with addition of 10^{-3} M SHAM (b), 10^{-4} M baicalein (c), 10^{-4} M esculetin (d), and 10^{-4} M NS398 (e). Incubation of 0.5 μCi [1-^{14}C]AA and the inhibitors with 0.5 ml of cell-free homogenates of the lung tissue was performed at 37°C for 30 min. Spots with the corresponding eicosanoids were cut off from the chromatograms and extracted with methanol for subsequent radiometry. The total radioactivity of the whole specimen and the relative radioactivity of each spot were measured as described in "Materials and Methods". The mean parameters (n = 3) of [^{14}C]AA conversion to 12-HETE/15-HETE and 5-HETE in the groups ± standard deviation are presented.

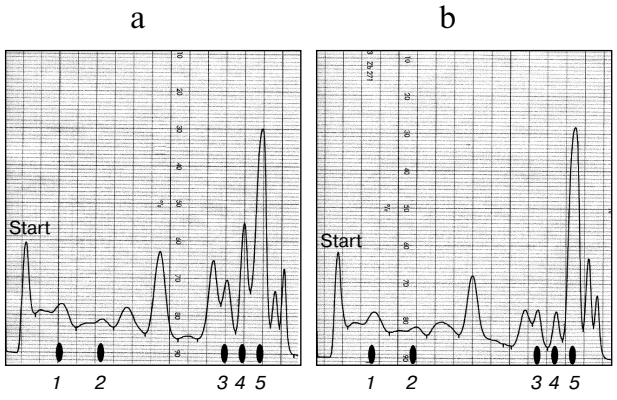

Fig. 2. Profiles of eicosanoids synthesized from exogenous [^{14}C]AA in control (a) homogenates of A431 cells of human epidermoid carcinoma and under the influence of the 12-LOX inhibitor, baicalein (b). Incubation of 0.5 μCi [1-^{14}C]AA and 10^{-5} M baicalein with 0.5 ml of cell-free homogenates of the tumor tissue was performed at 37°C for 30 min. Densitograms of radioautographs of thin layer chromatograms are presented. Positions of the standard spots are shown under the densitograms: *1*) $PGF_{2\alpha}$; *2*) PGE_2; *3*) 5-HETE; *4*) 12-HETE/15-HETE; *5*) AA.

The ratio of eicosanoids produced in the epidermoid carcinoma cells was completely different: 12-HETE/15-HETE >> 5-HETE > $PGF_{2\alpha}$ > PGE_2 (Fig. 2a). Thus, conversion of exogenous [^{14}C]AA to these metabolites in cell-free homogenates of tumor cells A431 was 10.44 ± 1.33, 5.38 ± 0.93, 5.20 ± 0.87, and 3.44 ± 0.54%, respectively. On HPLC analysis of the extract from the 12-HETE/15-HETE spot cut off from a TLC-plate, only 12-HETE was detected. Only 5-HETE and 12-HETE were found in the culture medium of the epidermoid carcinoma cells. Note that baicalein (10^{-5} M) significantly suppressed the synthesis of 12-HETE even after incubation for 30 min (3.19 ± 0.57%; –69% compared to the control), whereas the production of 5-HETE (3.85 ± 0.43%), $PGF_{2\alpha}$ (5.50 ± 0.61%), and PGE_2 (3.44 ± 0.47%) in A431 cells was affected insignificantly against the control (Fig. 2b). The same concentration of the inhibitor after prolonged (24 h) incubation with epidermoid carcinoma cells completely suppressed the synthesis of 12-HETE from incorporated [^{14}C]AA.

The ability of epidermoid carcinoma cells to incorporate [^{14}C]AA and also release labeled eicosanoids, including the unmetabolized precursor, into the culture medium markedly decreased with an increase in the cell growth density. In particular, the concentration of released labeled products of AA at the average growth density of the A431 culture of (46.60-82.80)·10^3 adhesive cells per cm^2 was 29.79-36.42 ng/ml, while at high density of culture (156.00·10^3 adhesive cells per cm^2) it was 9.58 ng/ml.

Effects of esculetin and baicalein on growth of A549 human adenocarcinoma cells. In the previous studies [11]

we have shown that in the 50%-confluent 4-day-old culture of A549 cells or at the early growth stages of the culture with the higher density, esculetin suppressed the incorporation of [^{3}H]thymidine into DNA significantly less effectively than indomethacin, but during the monolayer formation its efficiency decreased less markedly compared to the selective inhibitor of COX. Therefore, it was suggested that an important role in proliferation of A549 cells in the monolayer with the higher density should belong to 5-HETE, which, similarly to PGE_2, was synthesized in these cells in a great amount.

In the present work we studied the effect of esculetin on the incorporation of [^{3}H]thymidine into DNA and the growth of A549 culture at the density of (40-160)·10^3 adhesive cells per cm^2. With a fourfold increase in the culture density the esculetin-caused suppression of [^{3}H]thymidine incorporation into DNA decreased from –50 to –33% compared to the control on the second day of the growth and from –30 to –20% on the fourth day, and in some cases the decrease was to 0%. At the same time, the inhibitor of 12-LOX, baicalein, had no effect on the [^{3}H]thymidine incorporation into A549 cells without regard to the culture density and growth time. Note that the growth curves of tumor cells of this line were virtually the same in the control culture, which reached the monolayer, and in the cultures treated with esculetin and baicalein (Fig. 3).

Effects of inhibitors of AA metabolism on growth of A431 human epidermoid carcinoma cells. The epidermoid carcinoma cells were more sensitive to the inhibitor of 12-LOX, baicalein, than the cells of lung adenocarcinoma, and the effect was pronounced in both cultures not fully confluent on the fourth day (Fig. 4a) and in cultures with

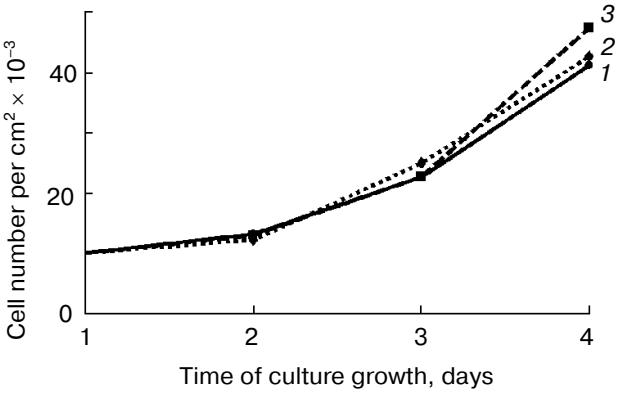

Fig. 3. Growth of A549 cells in control cultures (*1*) and those treated with 10^{-5} M baicalein (*2*) and 10^{-5} M esculetin (*3*). The inhibitors were added on the first, second, and third day of the culture growth simultaneously with changing of the medium. The culture seeding density was 6.3·10^4 cells per ml of the medium. The mean parameters (n = 3) of the growth density of the control and experimental cultures are presented (results of one of three independent experiments).

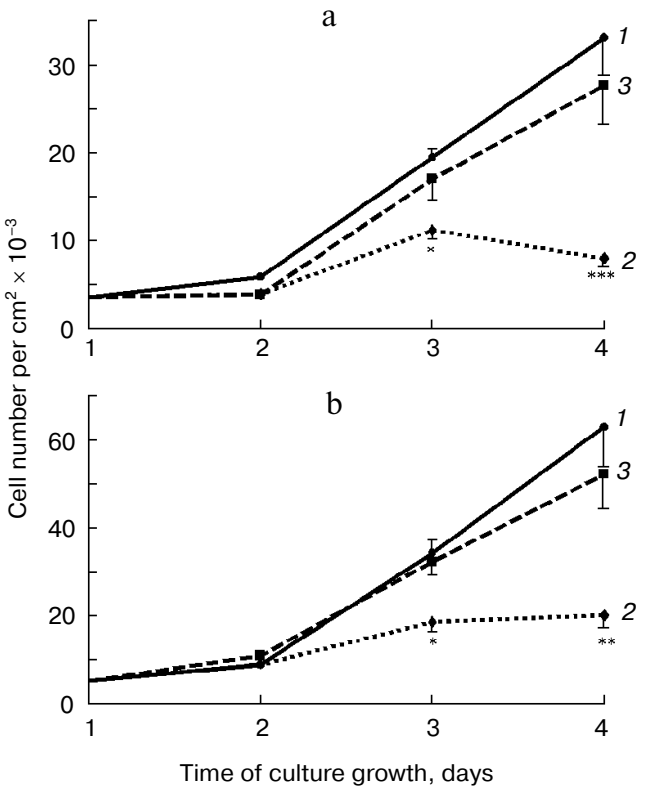

Fig. 4. Growth of A431 cells in control cultures (*1*) and under treatment with 10^{-5} M baicalein (*2*) and 10^{-5} M esculetin (*3*). The inhibitors were added on the first, second, and third day of the culture growth simultaneously with changing of the medium. The culture seeding density was 4.5·10^4 (a) and 5.0·10^4 (b) cells per ml of the medium. The mean parameters (n = 3) of the growth density of the control and experimental cultures ± the standard deviation are presented (results of one of three independent experiments). The statistical difference between parameters in the control and experimental groups: * $p < 0.01$; ** $p < 0.005$; *** $p < 0.002$.

the monolayer completed by the same time (Fig. 4b). In the first case, the quantity of adhesive cells in intact cultures of A431 cells on the fourth day of growth was (33.00 ± 2.99)·10^3 per cm^2 and the treatment with baicalein decreased it to (7.90 ± 0.61)·10^3 per cm^2 (–76% compared to the control; $p < 0.002$). At the higher density of growth, the number of adhesive cells in the control cultures was (62.80 ± 6.53)·10^3 per cm^2, and baicalein decreased it to (19.90 ± 2.03)·10^3 per cm^2 (–68%, $p < 0.005$). The effect of esculetin on this line of tumor cells was much weaker than the effect of baicalein. Esculetin decreased the number of adhesive cells in a non-confluent 4-day culture only to (27.60 ± 3.07)·10^3 per cm^2 (–16% compared to the control; Fig. 4a) and to (52.00 ± 5.57)·10^3 per cm^2 in the presence of the monolayer (–17% compared to the control; Fig. 4b).

Considering a slight growth-inhibiting effect of esculetin on A431 cells, it was important to assess it from the standpoint of a possible involvement of COX or 5-

LOX. On comparative study of the growth-modulating influences of AA metabolism inhibitors with different selectivity and efficiency we found that selective indomethacin and nonselective NS398 inhibitors of COX, which inhibited the biosynthesis of prostanoids in cell-free homogenates of lung tissue significantly stronger than esculetin, suppressed twofold stronger also the growth of the epidermoid carcinoma cells, including high density cultures ($160 \cdot 10^3$ adhesive cells per cm^2). Note that the growth inhibition with indomethacin (to –20% compared to the control) on the fourth day of the growth of the high density culture was manifested even on its single introduction on the third day, but a single introduction of baicalein was more effective (–45% compared to the control). It is important that inhibitors of 12-LOX and COX were also markedly different in their influence on the release of labeled eicosanoids into the culture medium. So, in contrast to the level of secretion of [^{14}C]AA metabolites in the control culture of A431 cells (3755 ± 337 cpm/ml), in the cultures treated with baicalein and indomethacin the label was decreased, respectively, to 2575 ± 241 (–31%; $p < 0.05$) and 3580 ± 363 (–5%) cpm per ml of the medium. The suppression of releasing of labeled eicosanoids into the culture medium under the influence of these inhibitors was accompanied by significant accumulation of the label in the tumor cells. The label concentration in the intact A431 cells and in those treated with baicalein and indomethacin was, respectively, 35.09 ± 4.01, 94.64 ± 9.73 (+170%; $p < 0.01$), and 66.35 ± 6.57 (+89%; $p < 0.02$) cpm per 10^3 adhesive cells.

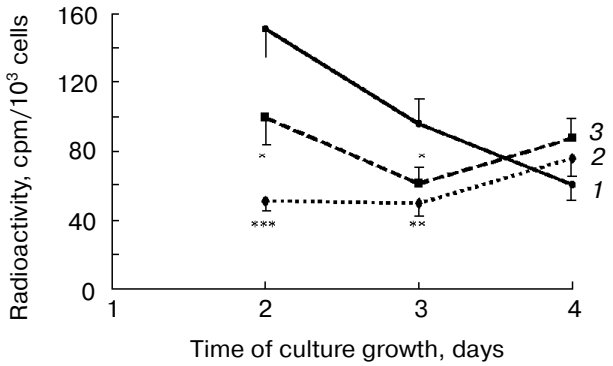

Fig. 5. Incorporation of [^{3}H]thymidine into A431 cells in control cultures (*1*) and those treated with 10^{-5} M baicalein (*2*) and 10^{-5} M esculetin (*3*). The inhibitors were added on the first, second, and third day of the culture growth simultaneously with changing of the medium. The culture seeding density was $5.0 \cdot 10^4$ cells per ml of the medium. [^{3}H]Thymidine (1 μCi/ml medium) was introduced as a 1-h pulse at 48, 72, and 96 h after beginning of the culture growth. The radioactivity of the cells was measured as described in "Materials and Methods". The mean radioactivity ($n = 3$) in the control and experimental cultures ± the standard deviation are presented (results of one of three independent experiments). The statistical difference between parameters in the control and experimental groups: * $p < 0.05$; ** $p < 0.005$; *** $p < 0.002$.

Studies on this line of the growth-modulating effect of SHAM, which is a powerful inhibitor of COX and various LOX, were especially interesting. This inhibitor suppressed by 60-65% the growth of A431 cell cultures, even at their high density, and this was only slightly less than under the influence of baicalein. These findings indicated that the inhibition of cyclooxygenase and lipoxygenase pathways of AA metabolism was no more efficient for the growth of A431 cells of human epidermoid carcinoma than the selective inhibition of 12-HETE synthesis.

Effects of inhibitors of AA metabolism on incorporation of [^{3}H]thymidine into DNA of A431 cells. Baicalein, an inhibitor of 12-LOX, effectively suppressed the incorporation of [^{3}H]thymidine into DNA of epidermoid carcinoma cells (Fig. 5). In the control cultures of A431 cells the label incorporation on the second day of the growth was 150.64 ± 12.02 cpm per 10^3 adhesive cells, and baicalein significantly decreased it to 50.71 ± 4.23 cpm per 10^3 adhesive cells (–66% compared to the control; $p < 0.002$). On the third day of growth these parameters in the control and treated cultures were, respectively, 95.69 ± 10.03 and 49.43 ± 5.13 cpm per 10^3 adhesive cells (–48% compared to the control; $p < 0.02$). As in the case of A549 cells treated with indomethacin, baicalein significantly reduced the incorporation of [^{3}H]thymidine into A431 cells during the early stages and its leveling was on the fourth day of the culture growth. Esculetin markedly less suppressed the [^{3}H]thymidine incorporation into the epidermoid carcinoma cells (Fig. 5). Under the influence of esculetin, the label incorporation into DNA on the second and third days of the culture growth was, respectively, 99.21 ± 10.87 (–34% compared to the control; $p < 0.05$) and 61.01 ± 6.33 (–36%; $p < 0.05$) cpm per 10^3 adhesive cells.

Growth-modulating effects of the main lipoxygenase metabolites of AA on A431 human epidermoid carcinoma cells. Unlike the expressed growth-inhibiting effect of 15-HETE on A549 cells [11], this lipoxygenase metabolite of AA, similarly to 5-HETE, had no significant influence on the growth and incorporation of [^{3}H]thymidine into DNA of A431 cells. The stimulation of the epidermoid carcinoma cell growth with 15-HETE and its suppression with 5-HETE were generally no more than 10%, whereas addition of exogenous 12-HETE (10 ng/ml) into the medium induced a stimulation of A431 cells to 33% compared to the control. Moreover, on the background of the suppressed synthesis of endogenous 12-HETE and significant inhibition of proliferation with baicalein, the injection of this eicosanoid into the culture virtually restored the growth potential of the tumor cells, and this effect was observed even at high density of the culture growth (Fig. 6). Thus, on the fourth day of growth the number of adhesive A431 cells in the presence of baicalein was $(56.10 \pm 6.47) \cdot 10^3$ per cm^2, and the combined introduction of the

12-LOX inhibitor with 12-HETE increased this parameter to $(100.00 \pm 9.89) \cdot 10^3$ per cm^2 (+78% compared to the effect of the inhibitor alone, $p < 0.05$). Note that other mono-HETE did not abolish the growth-inhibiting effect of baicalein, 15-HETE decreased it only by 15%, and 5-HETE increased it insignificantly (by 4%).

Thus, baicalein, an inhibitor of 12-LOX, markedly suppressed the proliferation and also lowered to 70% the incorporation of [^{3}H]thymidine into DNA of A431 epidermoid carcinoma cells which have 12-HETE as a dominating lipoxygenase metabolite of AA among the synthesized eicosanoids. The addition of 12-HETE into these cultures virtually restored the growth potential of the tumor cells. Inhibitors of COX were much less efficient in suppression of the growth of epidermoid carcinoma cells. The findings suggest that in tumor cells of a definite type the main growth-limiting factor is not PGE_2 but 12-HETE, a representative of another, lipoxygenase pathway of AA metabolism.

Fig. 6. Effect of exogenous 12-HETE on the growth of A431 cells under conditions of 12-LOX inhibition with baicalein. *1*) Control; *2*) baicalein; *3*) 12-HETE + baicalein. Baicalein (10^{-5} M) and 12-HETE (10 ng/ml) were added on the first, second, and third day of the culture growth simultaneously with changing of the medium. The culture seeding density was $6.0 \cdot 10^4$ cells per ml of the medium. The mean parameters ($n = 3$) of the growth density in the control and experimental cultures ± the standard deviation are presented (results of one of three independent experiments). The difference between the parameters in the second and third groups on the fourth day of the growth is statistically significant ($p < 0.05$).

DISCUSSION

Contrastingly to other known eicosanoids, the major cyclooxygenase metabolite of AA, PGE_2, is usually considered to be mainly a procarcinogenic agent. PGE_2 can induce and mediate expression of some oncogenes [25, 26], display a strong immunosuppressive effect [27], and it is an important factor for angiogenesis [28, 29], growth [7-9], and metastasizing [30] of tumors. On the other hand, some oncogenes, chemical carcinogens, promoters, and various factors essential for carcinogenesis are inducers of COX and, as a consequence, the biosynthesis of PGE_2 [2, 3, 31]. Very high levels of biosynthesis of this prostanoid are characteristic for many malignant tumors of humans and animals [32, 33]. However, application of active inhibitors of COX-1 and COX-2 do not always result in an anticarcinogenic effect [34, 35] or inhibition of the growth of experimental tumors [36, 37]. A reasonable question is whether lipoxygenase metabolites of AA can limit the growth of such tumors. Recent investigations have shown that tumors of different histogenesis considerable differ in metabolism of AA, in particular there are tumors with a preferential synthesis of 12-HETE/15-HETE [9]. In this context, studies on the role of different mono-HETE in the growth control of tumor cells are of crucial importance.

There are also some recent data about the growth-stimulating effect of 5-HETE on MCF-7 mammary gland carcinoma cells and PC-3 human prostate cancer cells and also about high efficiency of 5-LOX inhibitors in suppression of their growth [13-17]. In contrast, 15-HETE, another lipoxygenase metabolite of AA, has been shown to inhibit the PGE_2-dependent proliferation and to be accumulated in the medium during the growth of tumor cell culture [11].

We have shown that the 12-LOX inhibitor, baicalein, very effectively suppressed the growth of A431 cells of human epidermoid carcinoma and pronouncedly lowered the incorporation of [^{3}H]thymidine into DNA. Note that the effect of baicalein on the label incorporation into A431 cells was similar to the effect of indomethacin on the [^{3}H]thymidine incorporation into A549 lung adenocarcinoma cells, in which the activity of the COX inhibitor also was the highest on the early stages of growth or at low density of cultures [11]. The addition of exogenous 12-HETE to the baicalein-treated cultures virtually restored the growth potential of A431 cells. However, 15-HETE, which markedly inhibited the growth of A549 cells, and also 5-HETE had no influence on the epidermoid carcinoma cells. These findings suggest that a dominating role in proliferation of the A431 human epidermoid carcinoma cells is played by 12-LOX, which is one of the directions of the lipoxygenase pathway of AA metabolism.

Certainly, there is an essential question whether other pathways of eicosanoid production, apart from 12-LOX, in particular COX and 5-LOX, are important for the control of the growth of A431 cells. In this connection, the activity of COX inhibitors in suppression of the growth of epidermoid carcinoma cells is especially interesting. Although their growth-inhibiting effect was significantly lower than that of baicalein, it was constant and manifested itself even on a single introduction. The ability of the COX inhibitor indomethacin to suppress the growth of A431 cells was also found by other authors [38]. An implication of such an "auxiliary" role of the

cyclooxygenase pathway of AA metabolism for proliferation of A431 cells is yet unclear.

Both 5-LOX and COX-1, unlike 15-LOX, COX-2, and especially 12-LOX, are more often expressed in tumor cells [19]. Nevertheless, the role of 5-LOX in the growth control of A549 and A431 cells seems unimportant, because the studied nonselective inhibitors of AA metabolism display a significantly lower growth-inhibiting effect than indomethacin or baicalein, and the introduction of 5-HETE was ineffective in all cases. Therefore, attention should be paid to assessment of the growth-modulating effect on these tumor cell lines of such an inhibitor of AA metabolism as esculetin, which was shown to equally inhibit the synthesis of both prostanoids and 5-HETE. The inhibitory effect of esculetin on the growth of tumor cells, especially adenocarcinoma cells, was less pronounced than its suppressing effect on the [^{3}H]thymidine incorporation. It seems that such an unusual effect of esculetin is caused by its influence on both pathways of AA metabolism, and the influence on 5-LOX is likely to be associated not with proliferation of the tumor cells but with their death. This is in agreement with our data that esculetin significantly decreased the survival of A549 and A431 cells when tested with Trypan Blue.

Thus, depending on the tumor cell type, both cyclooxygenase and lipoxygenase AA metabolites, such as PGE_2, 5-HETE, and 12-HETE, can be growth-limiting factors. The essential importance of both pathways of AA metabolism is a prerequisite for studies in detail of individual eicosanoids and inhibitors of their synthesis in processes associated with cell death, including apoptosis. Domination of different pathways of AA metabolism in proliferation processes of tumor cells of different histogenesis must be taken into account on consideration of a possible addition of cyclooxygenase and lipoxygenase inhibitors to schemes of antitumor therapy.

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