Dual inhibition of 5-LOX and COX-2 suppresses colon cancer formation promoted by cigarette smoke


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Abbreviations: LOX, lipoxygenase; COX, cyclooxygenase; PG, prostaglandin; LT, leukotriene; CSE, cigarette smoke extract; 5(S)-HETE, 5(S)-hydroxy-eicasoetraenoic acid.

Previous studies indicate that the arachidonic acid-metabolizing enzymes COX-2 and 5-LOX are overexpressed during the process of colonic adenoma formation promoted by cigarette smoke. The aims of the present study were to investigate whether there exists a relationship between COX-2 and 5-LOX, and whether dual inhibition of COX-2 and 5-LOX has an anticarcinogenic effect in the colonic tumorigenesis promoted by cigarette smoke. Results showed that pretreating colon cancer cells with cigarette smoke extract (CSE) promoted colon cancer growth in the nude mouse xenograft model. Inhibition of COX-2 or 5-LOX reduced the tumor size. In the group treated with COX-2-inhibitor, the PGE2 level decreased while the LTB4 level increased. In contrast, in the 5-LOX-inhibitor treated group, the LTB4 level was reduced and the PGE2 level was unchanged. However, combined treatment with both COX-2 and 5-LOX inhibitors further inhibited the tumor growth promoted by CSE over treatment with either COX-2-inhibitor or 5-LOX-inhibitor alone. This was accompanied by the downregulation of PGE2 and LTB4. In an in vitro study, we found that the action of CSE on colon cancer cells was mediated by 5-LOX DNA demethylation. In summary, these results indicate that inhibition of COX-2 may lead to a shunt of arachidonic acid metabolism towards the leukotriene pathway during colonic tumorigenesis promoted by CSE. Suppression of 5-LOX did not induce such a shunt and produced a better response. Therefore, 5-LOX inhibitor is more effective than COX-2 inhibitor, and blocker of both COX-2 and 5-LOX may present a superior anticancer profile in cigarette smokers.
formation promoted by cigarette smoke and have been implicated as mediators of tumor development (17,18). The question is whether they coregulate or act independently in the process of tumor growth. The aims of the present study were to investigate whether there exists a relationship between COX-2 and 5-LOX, as well as to determine the anticarcinogenic effect of dual inhibition of COX-2 and 5-LOX in the colonic tumorigenesis promoted by cigarette smoke.

Materials and methods

Cell culture and chemicals
SW1116, a cell line derived from human colon adenocarcinoma, was obtained from the American Type Culture Collection (Manassas, VA) and cultured in Leibovitz’s L-15 medium containing 10% (v/v) heat-inactivated fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Chemicals were purchased from Sigma-Aldrich (St Louis, MO) unless otherwise stated. 5-LOX monoclonal antibody, F58420, was from BD Transduction Laboratories (Lexington, KY). COX-2 polyclonal antibody, SC-7951, and β-actin antibody were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA) and Sigma (AC-15), respectively.

Cigarette smoke extract (CSE) preparation
The commercial cigarette brand Camel™ (R.J. Reynolds, Winston-Salem, NC, USA) was used in the present study. CSE was prepared by passing the cigarette smoke through four successive flasks of 95% ethanol followed by two flasks of absolute chloroform (19). The ethanol- and chloroform-soluble fractions were concentrated by evaporation and then combined as expressed as CSE. This extract contained the major part of components in cigarette smoke (20).

Cell proliferation assay
Cell proliferation was assessed as DNA synthesis. To evaluate DNA synthesis in cells, the incorporation of [3H]thymidine into DNA was determined. Briefly, cells were seeded into a 24-well plate and cultured for 24 h for attachment. They were then washed twice with 0.01 M phosphate-buffered saline (PBS), followed by incubation with 1 ml/well of the medium containing certain substances for 5 h. In the next step, 0.5 μCi of [3H]thymidine was added to each well and the cells were further incubated for 5 h. Incorporation of [3H]thymidine into cells was measured with a liquid scintillation counter (LS-6500; Beckman Instruments, Inc.).

Tumorigenicity in nude mice
Suspensions of SW1116 cells were trypsinized and collected. The cell viability was >95% as determined by trypan blue staining. Cells (3 x 10⁶) in a 0.2 ml volume of PBS were inoculated subcutaneously into the right flank of 4-week-old female BALB/c nu/nu mice (Laboratory Animal Unit, The University of Hong Kong). The mice were maintained under sterile conditions. Tumor sizes were determined by measuring two diameters perpendicular to each other with a caliper every 3 days. Tumor volume (V) was estimated by using the equation $V = \frac{1}{2} \times \frac{3}{4} \times \frac{d^2}{2} \times \frac{d_2}{2}$, where L is the mid-axis length and W is the mid-axis width. At the end of the experiments, the mice were killed and the tumors were excised and kept in 4% formalin or liquid nitrogen for assays.

Immunohistochemical determination of cellular proliferation, apoptosis and angiogenesis in tumors

The procedure for the determination of cell proliferation was described previously (21). Briefly, after heating tissue sections in citrate buffer (0.01 M, pH 6.0) at 80°C for 15 min, they were subjected to pepsin (0.005% HCl) (0.01 N, pH 2.0) digestion. After blocking with normal serum, the monoclonal antiproliferating cell nuclear antigen (PCNA) antibody (1:200) was applied to the sections for 2 h at room temperature. The antibody-labeled cells were visualized by using the labeled streptavidin–biotin DAKO kit (Dako, Glostrup, Denmark), in combination with 3,3′-diaminobenzidine (DAB, 0.4 mg/ml). The number of proliferating cells was recorded in 6–8 randomized fields (x400) under the microscope and was expressed as the number per two fields. Apoptotic cells were visualized by the terminal deoxytransferase (TdT)-mediated dUTP nick end labeling (TUNEL) method (22). Briefly, tissue sections were subjected to digestion by protease K. TdT buffer solution [30 mM Tris–HCl (pH 7.0) with 140 mM sodium cacodylate, 1 mg/ml BSA and 1 mM CoCl₂] was added together with 50 U TdT and 5 mM dUTP to the tissue sections and incubated at 37°C for 90 min. Then the reaction was terminated by 30 mM sodium citrate buffer. Peroxidase-conjugated streptavidin from the DAKO kit was added and this was followed by the addition of DAB. The number of apoptotic cells was counted under a microscope (x400) and was expressed as the number per two fields.

Microvessels in the colonic mucosa were identified by staining with the von Willebrand factor antibody (23). Tissue sections were incubated with 0.3% H₂O₂–methanol for 15 min, followed by trypsin (1 mg/ml) digestion for 30 min. After blocking with normal serum, sections were incubated overnight with the von Willebrand factor antibody (1:200). On the following day, the DAKO kit in combination with DAB was used to visualize endothelial cells of the blood vessels. The number of blood vessels was counted in six randomized fields (x200) in the tumor tissue using a light microscope, and expressed as number per mm².

Western-blot analysis
The cells and the tumors were harvested at 4°C with RIPA buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 0.5% ω-cholate, 0.1% SDS, 2 mM EDTA, 1% Triton X-100 and 10% glycerol) containing 1.0 mM phenylmethylsulfonyl fluoride and 1 μg/ml aprotinin. After sonication or homogenization on ice, the samples were centrifuged at 17,968 × g for 20 min at 4°C and the supernatant containing 70 μg of the protein was denatured and separated by electrophoresis on a sodium dodecyl polyacrylamide gel. The protein was then transferred to a nitrocellulose membrane (Bio-Rad, Hercules, CA, USA) that was probed with the respective primary antibody. Membranes were developed by the ECL chemiluminescence and exposed on X-ray film. Quantification of the bands on the film was carried out by video densitometry (Gel Doc 1000, Bio Rad).

Measurement of LTB₄ and PGE₂
The tumor tissues and cell samples were harvested with Tris–HCl buffer (50 mMol/l, pH 7.4) containing 100 mMol/l NaCl, 1 mMol/l CaCl₂, 1 mg/ml t-glucuronide and 28 μmol indomethacin. The homogenate was centrifuged at 15,000 for 15 min at 4°C. The supernatant was assayed using LTB₄ and PGE₂ immunoassay kits (R & D Systems, Minneapolis, MN). The samples were measured with a microplate reader (Dynex Technologies, Microtiter Co., Chantilly, VA) at 405 nm. A standard curve for LTB₄ assay was obtained in the range of 46.9–12,000 pg/ml, while a standard curve for PGE₂ assay was obtained in the range of 39–5000 pg/ml. The protein content assay was based on the Dye-reagent method using a standard protein (bovine albumin, 10 g/l) as standard. The final values for the samples are expressed as picograms per milligram of protein.

DNA isolation
Genomic DNA was isolated by proteinase K digestion and a 2-fold phenol/chloroform/soyamylalcohol extraction. After precipitation by the addition of 2 volumes of ethanol and 0.5 volume of ammonium acetate (7.5 M), the DNA was washed twice with ethanol (80%, v/v) and dissolved in water.

Methylation-specific PCR
DNA methylation patterns in the 5-LOX promoter were determined by methylation-specific PCR, as described by Tsuchiya et al. (24–26) with some modifications. This method distinguishes unmethylated alleles from methylated alleles in a given gene based on sequence changes produced after bisulfite treatment of DNA, which converts unmethylated but not methylated cytosines to uracil, and subsequent PCR using primers specific to either methylated or unmethylated DNA. One microgram of genomic DNA was bisulfite modified (CpGenome DNA modification kit; Intergen Co., Purchase, NY). The PCR was performed by using primer pairs described below with the following conditions. In 50 μl, the PCR mix contained 1× PCR buffer [16.6 mM ammonium sulfate, 67 mM Tris (pH 8.8), 6.7 mM MgCl₂ and 10 mM 2-mercaptoethanol], deoxynucleotide triphosphates (each 1.25 mM), primers (20 pmol each of sense and antisense primers), bisulfite-modified DNA (50 ng) and 1.5 U of platinum Taq DNA polymerase (Life Technologies, Inc., Rockville, MD). Amplification was carried out for 32 cycles (30 s at 95°C, 30 s at 66°C and then 30 s at 72°C, followed by a final 4-min extension at 72°C). The PCR reaction products were loaded onto nondenaturing 6% polyacrylamide gels, stained with ethidium bromide and visualized under UV illumination. 5-LOX primer sequences were designed based on the published promoter sequence (27) as follows: unmethylated reaction, 5'-AACAGCTTGGGGTTGGGCTTGGACT-3' (sense) and 5'-ACAGGCCCAACAGAGCCAGCAG-CAAG-3' (antisense); and methylated reaction, 5'-AACATCTCGGGGTGGGCTTGGACT-3' (sense) and 5'-ATCACGGCAGAAGAGCCAGCCGACG-3' (antisense).

Statistical analysis
Student’s t-test was used to compare data between two groups. One-way ANOVA and the Bonferroni correction were used to compare data between three or more groups. Values are expressed as mean ± SEM. P < 0.05 was considered statistically significant.
Colon cancer and cigarette smoke

Table I. Measurement of cell proliferation, apoptosis and angiogenesis in the colonic tumor tissues in the nude mouse xenograft model

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of proliferating cells/two fields</th>
<th>Number of apoptotic cells/two fields</th>
<th>Ratio of apoptosis over proliferation</th>
<th>Number of blood vessels/mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 10 (Ctrl)</td>
<td>25.41 ± 1.98</td>
<td>6.12 ± 0.29</td>
<td>0.243 ± 0.015</td>
<td>2.18 ± 0.32</td>
</tr>
<tr>
<td>Day 10 (CSE)</td>
<td>36.79 ± 2.13b</td>
<td>9.32 ± 0.37b</td>
<td>0.261 ± 0.017</td>
<td>2.07 ± 0.35</td>
</tr>
<tr>
<td>Day 16 (Ctrl)</td>
<td>27.02 ± 1.76</td>
<td>6.52 ± 0.23</td>
<td>0.241 ± 0.013</td>
<td>2.39 ± 0.27</td>
</tr>
<tr>
<td>Day 16 (CSE)</td>
<td>42.63 ± 2.17b</td>
<td>8.06 ± 0.28a</td>
<td>0.190 ± 0.013</td>
<td>3.42 ± 0.26a</td>
</tr>
<tr>
<td>Day 27 (Ctrl)</td>
<td>29.13 ± 2.06</td>
<td>6.84 ± 0.31</td>
<td>0.235 ± 0.015</td>
<td>3.22 ± 0.29</td>
</tr>
<tr>
<td>Day 27 (CSE)</td>
<td>53.57 ± 2.25b</td>
<td>7.36 ± 0.25</td>
<td>0.142 ± 0.011b</td>
<td>5.17 ± 0.32b</td>
</tr>
</tbody>
</table>

SW1116 cells were pretreated with 100 μg/ml CSE for 5 h before inoculation into the nude mice. Values were expressed as mean ± SEM of six independent experiments.

*p<0.05.

*p<0.01 when compared with the corresponding control (Ctrl) group.

Results

Effect of CSE on colon cancer growth

Pretreatment of SW1116 cells with CSE for 5 h before inoculation into the nude mice promoted colon cancer growth in this xenograft model in a dose-dependent manner. Interestingly, the tumor growth rates were identical up to 16 days postinjection and then differed from that point up to the end of the experiment at 27 days (Figure 1). On day 27, the tumor sizes from cells exposed to the three doses of CSE were significantly greater than the control. The highest dose of CSE (100 μg/ml) produced a 3-fold increase in tumor size (Figure 1).

Levels of cell proliferation, apoptosis and angiogenesis in colon cancer xenograft

On day 10 after colon cancer cell implantation, when the tumor size was identical for all groups, immunohistological analysis indicated that both cell proliferation and apoptosis, but not angiogenesis, were significantly enhanced in the tumors from cells pretreated with 100 μg/ml CSE compared to the control group. On days 16 and 27, the CSE-pretreated cells proliferated more rapidly. This was accompanied by an increased neovascularization and reduction in apoptotic index (Table I). The ratio of apoptosis to proliferation showed no significant difference between the CSE pretreatment and control on the 10th day. However, on the 16th and 27th days, the ratio significantly decreased in the tumors from cells pretreated with CSE (Table I).

Effects of CSE on COX-2, 5-LOX, PGE2 and LTB4 levels in tumors

Western-blot analysis showed that the protein expressions of 5-LOX and COX-2 levels were higher in tumors derived from CSE-treated cells 10 days after implantation and such levels were maintained to the end of the experimental period (Figure 2A). The levels of LTB4 and PGE2 in tumor tissues from CSE-treated cells were significantly upregulated on the 10th, 16th and 27th days (Figure 2B and C).

Effects of pharmacological inhibitors of COX-2 and 5-LOX on tumor growth promoted by CSE

After colon cancer cell implantation, the pharmacological inhibitors of COX-2 (celecoxib: 12.5 and 25 mg/kg, i.p.) and/or 5-LOX (AA861: 12.5 and 25 mg/kg, p.o.) were given to the nude mice every other day. By day 27 after implantation, both inhibitors of COX-2 and 5-LOX had significantly reduced the stimulatory action of CSE on tumor size in the xenograft model. Celecoxib caused 30% reduction of tumor growth, while AA861 caused 50% inhibition relative to the control group. However, there were no significant differences between the higher and lower doses of the two inhibitors (Figure 3).

Interestingly, when celecoxib and AA861 were combined at their lower doses, the inhibitory action was more pronounced than treatment with either inhibitor alone (Figure 3). In addition, immunohistological study showed that the cell proliferation and angiogenesis induced by CSE were also inhibited by celecoxib and AA861, either separately or in combination. Moreover, dual inhibition of COX-2 and 5-LOX by celecoxib and AA861 produced greater response than the individual inhibitors (Table II).

Effects of celecoxib and AA861 on PGE2 and LTB4 levels in tumors promoted by CSE

By day 27 after colon cancer cell implantation, PGE2 and LTB4 levels significantly increased in the tumors derived from cells exposed to CSE. Celecoxib (12.5 mg/kg) decreased the PGE2 level by 16.3% while the LTB4 level increased ~125% in these tumors. In the AA861-treated group (12.5 mg/kg), the LTB4 level was reduced by 34.7% and the PGE2 level was unchanged. Combined treatment with celecoxib and AA861 further downregulated both PGE2 and LTB4 levels in the tumor tissues (Figure 4).
Effects of CSE on colon cancer cells in vitro study

Our results above showed that pretreating SW1116 cells with CSE for 5 h before inoculation into nude mice promoted tumor growth in a dose-dependent manner (Figure 1). In this step, we further investigated how CSE affected colon cancer cells during the 5 h incubation. We found neither an increase in COX-2 expression nor any effect on PGE2 level in the SW1116 cells. However, the production of 5-LOX and LTB4 was enhanced by CSE (Figure 5A and C). In addition, we also

Table II. Effects of inhibitors of COX-2 and/or 5-LOX on cell proliferation, apoptosis and angiogenesis in the tumor tissues in the nude mouse xenograft model on day 27 after colon cancer cell implantation

<table>
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<th>Groups</th>
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<td>3.22 ± 0.29</td>
</tr>
<tr>
<td>CSE</td>
<td>53.55 ± 2.25c</td>
<td>7.36 ± 0.25</td>
<td>0.142 ± 0.011c</td>
<td>5.17 ± 0.32c</td>
</tr>
<tr>
<td>CSE+Cele</td>
<td>46.27 ± 1.99b,c</td>
<td>7.24 ± 0.23</td>
<td>0.156 ± 0.012c</td>
<td>4.56 ± 0.24b,c</td>
</tr>
<tr>
<td>CSE+AA</td>
<td>38.91 ± 2.32a,b</td>
<td>7.52 ± 0.27</td>
<td>0.194 ± 0.013a,b</td>
<td>4.35 ± 0.25b,c</td>
</tr>
<tr>
<td>CSE+Cele+AA</td>
<td>33.87 ± 2.18a,b</td>
<td>7.19 ± 0.33</td>
<td>0.212 ± 0.015a,b</td>
<td>3.76 ± 0.21a,b</td>
</tr>
</tbody>
</table>

SW1116 cells were pretreated with 100 μg/ml CSE for 5 h before inoculation into the nude mice. After cell implantation, celecoxib (Cele: 12.5 mg/kg, i.p.) and/or AA861 (AA: 12.5 mg/kg, p.o.) were given to the nude mice every other day. Data are expressed as mean ± SEM of six independent experiments.

*P<0.05 when compared with the corresponding control (Ctrl) group.

P<0.05 when compared with the corresponding CSE group.

P<0.05 when compared with the ‘CSE+Cele+AA’ group.

Fig. 2. Protein expressions of 5-LOX and COX-2 (A) and the levels of LTB4 (B) and PGE2 (C) in tumor tissues in nude mouse xenograft model at different stages. Before inoculation into the nude mice, SW1116 cells were pretreated with 100 μg/ml CSE or control medium for 5 h. (A) Representative western blot results from six separate experiments. (B) Data are expressed as mean ± SEM of six independent samples (n=18). *, P<0.05 compared with the corresponding control group.

Effects of CSE on colon cancer cells in vitro study

Our results above showed that pretreating SW1116 cells with CSE for 5 h before inoculation into nude mice promoted tumor growth in a dose-dependent manner (Figure 1). In this step, we further investigated how CSE affected colon cancer cells during the 5 h incubation. We found neither an increase in COX-2 expression nor any effect on PGE2 level in the SW1116 cells. However, the production of 5-LOX and LTB4 was enhanced by CSE (Figure 5A and C). In addition, we also

Fig. 3. Effects of pharmacological inhibitors of COX-2 and 5-LOX on tumor growth promoted by CSE. SW1116 cells were pretreated with 100 μg/ml CSE for 5 h before inoculation into the nude mice. After cell implantation, celecoxib (Cele: 12.5 and 25 mg/kg, i.p.) and AA861 (AA: 12.5 and 25 mg/kg, p.o.) were given to the mice every other day. Data are expressed as mean ± SEM of six independent samples (n=18). *, P<0.05 compared with the control group; #, P<0.05 compared with the CSE group; $, P<0.05 compared with the ‘CSE+Cele12.5+AA12.5’ group.

Fig. 4. Effect of celecoxib and AA861 on PGE2 and LTB4 levels in tumors promoted by CSE. SW1116 cells were pretreated with 100 μg/ml CSE for 5 h before inoculation into the nude mice. After implantation, celecoxib (Cele: 12.5 mg/kg, i.p.) and AA861 (AA: 12.5 mg/kg, p.o.) were given to the mice every other day. Data are expressed as mean ± SEM of six independent samples (n=18). *, P<0.05 compared with the corresponding control group; #, P<0.05 compared with the CSE group.
found that the cell proliferation and vascular endothelial growth factor (VEGF) were significantly promoted by CSE after 5 h of incubation (Figure 5D).

Effect of CSE on 5-LOX gene promoter methylation in tumor cells

As there was a marked upregulation of 5-LOX expression in SW1116 cells treated with CSE, we assessed the 5-LOX gene promoter methylation status in these cells. We identified a CpG-dense region at nucleotides 103--121 of the 5-LOX gene promoter before the transcription start site. Therefore, primers for methylation-specific PCR were designed that identified unmethylated or methylated DNA sequences within this region of the promoter. As shown in Figure 5B, a strongly-methylated product was evident under basal conditions. In contrast, the cells treated with CSE for 5 h exhibited a marked increase in unmethylated 5-LOX promoter.

Temozolomide (TMZ, 100 μM), a DNA methylating agent, reversed the action of CSE by increasing the methylation in the 5-LOX promoter (Figure 5B). The increased 5-LOX protein expression and LTB4 level were also downregulated by TMZ without affecting the COX-2 and PGE2 levels (Figure 5A and C). The increased cell proliferation and VEGF protein expression promoted by CSE were nearly completely blocked by TMZ (Figure 5D).

Discussion

In the absence of angiogenesis, tumor growth is restricted to a maximum size of 1--2 mm in diameter. Up to this size, tumor cells can obtain the oxygen and nutrients necessary for growth and survival by simple passive diffusion (28). On the other hand, tumor cell proliferation is balanced by high rates of apoptosis (29). In such a condition the tumor will not grow further as long as it remains non-angiogenic. These small tumor masses can eventually induce angiogenesis by recruiting surrounding mature host blood vessels to generate new capillaries that grow toward and infiltrate the tumor mass, setting in motion the potential for expansion and hematogenous metastatic spread. This angiogenic process needs a relatively longer period of time when compared with cell proliferation and apoptosis, usually from several days to several months (30, 31). In the present study, we found that tumors grew at a similar rate on day 10 when angiogenesis was not affected by CSE. It was then, from 16 to 27 days, that the cells pretreated with CSE proliferated more rapidly along with a marked increase in blood vessel number in the tumor tissues (Figure 1). These findings suggest that the stimulatory action of some of the angiogenic factors such as VEGF on angiogenesis, which was activated by CSE (Figure 5D), would only be reflected at the later stage of tumor growth. This delayed response has indeed concurred with previous studies (30, 31). The early expression of angiogenic factors is important to initiate the angiogenic process to maintain tumor growth at the later stage of cancer development. Our data strongly support this scenario. In the early phase, the rapid cell proliferation promoted by CSE was accompanied by increased apoptosis, but not by angiogenesis. In the later stage, the increased cell proliferation was consistent with the upregulation of angiogenesis (Table 1). These findings would suggest that the increase of tumor growth by CSE would be highly dependent on angiogenesis initiated by angiogenic factors in tumor tissues.

The arachidonic acid-metabolizing enzymes, COX-2 and 5-LOX, are frequently overexpressed in human cancers and have been implicated as mediators of tumor development. Our results showed that inhibition of COX-2 and 5-LOX by pharmacological inhibitors reduced the colon cancer growth by 30 and 50%, respectively, in our nude mouse xenograft model. In these drug-treated mice, neither physical changes nor abnormalities in food and water consumptions were observable. In the group treated with the COX-2 inhibitor, the PGE2 level decreased by 16.3% while the LTB4 level increased by 125% relative to the group inoculated with CSE pretreated cells, and yet the tumor size was significantly reduced by celecoxib suggesting that COX-2 metabolites, in addition to PGE2, could also be involved in the promotion of colon cancer by CSE.
In the group treated with AA861, the LTB₄ level was reduced by 60% and the PGE₂ level did not change. However, combined treatment with the COX-2 and 5-LOX inhibitors further inhibited the tumor growth promoted by CSE over the treatment with either celecoxib or AA861 alone. This dual treatment was accompanied by the downregulation of PGE₂ and LTB₄ in the tumor tissues. Therefore, our data suggested that the single use of COX-2 or 5-LOX inhibitors might be an inefficient means of suppressing tumor growth. In cancers coexpressing COX-2 and 5-LOX, their simultaneous inhibition may prevent the shunting of arachidonic acid toward 5-LOX when COX-2 is blocked, thus suppressing the production of 5-LOX-derived mitogenic proangiogenic eicosanoids. Therefore, the discovery of compounds that can inhibit both the main metabolic pathways of the arachidonic acid metabolism would improve the efficacy and reduce side effects as lower doses are being used and this is of considerable interest (32).

It was found that COX-2 expression was progressively induced during the tumor development, while 5-LOX was increased 10 days after cancer cell implantation and found to be consistent thereafter to the end of the experiment (Figure 2A). However, the LTB₄ production kept increasing during the tumor development (Figure 2B). This finding can be explained by the fact that LTB₄ exerts a positive feedback mediated by its receptors and downstream signaling molecules (33,34). In addition, celecoxib and AA861 did not affect apoptosis in the tumor cells but inhibited tumor cell proliferation in vivo. This is quite different when compared with those found in vitro studies, in which both celecoxib and AA861 can induce apoptosis in different tumor cells (35,36). The discrepancy could be due to the differences between isolated cell preparation and whole animal model, which may have different signal transduction pathways and biological outcomes in apoptosis.

The cellular origin of COX-2 overexpression in tumors is a matter of debate. Cancer cells, non-transformed epithelial cells, stromal fibroblasts, vascular endothelial cells and inflammatory infiltrates are constituents of neoplastic colon tissue, and all of these cells are reported to display elevated inflammatory infiltrates are constituents of neoplastic colon cells, stromal fibroblasts, vascular endothelial cells and is a matter of debate. Cancer cells, non-transformed epithelial cells, stromal fibroblasts, vascular endothelial cells and inflammatory infiltrates are constituents of neoplastic colon tissue, and all of these cells are reported to display elevated inflammatory infiltrates are constituents of neoplastic colon cells, stromal fibroblasts, vascular endothelial cells and inflammatory infiltrates are constituents of neoplastic colon cells, stromal fibroblasts, vascular endothelial cells and inflammatory infiltrates are constituents of neoplastic colon cells, stromal fibroblasts, vascular endothelial cells and inflammatory infiltrates are constituents of neoplastic colon cells, stromal fibroblasts, vascular endothelial cells and inflammatory infiltrates are constituents of neoplastic colon cells, stromal fibroblasts, vascular endothelial cells and inflammatory infiltrates are constituents of neoplastic colon cells, stromal fibroblasts, vascular endothelial cells.

References

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