Biochemical and Biophysical Research Communications 402 (2010) 308–311

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Role of leukotriene B_4 in celecoxib-mediated anticancer effect

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ARTICLE INFO

Article history:
Received 26 September 2010
Available online 19 October 2010

Keywords:
Celecoxib
Leukotriene B_4
Colon cancer
Prostate cancer

ABSTRACT

Celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor, has anticancer effect on many cancers associated with chronic inflammation by both COX-2-dependent and COX-2-independent mechanisms. The non-COX-2 targets of celecoxib, however, are still a matter of research. Leukotriene B_4 (LTB_4) has been implicated in prostate and colon carcinogenesis, but little is known about the potential role of LTB_4 in celecoxib-mediated anticancer effect. In this study, we evaluated whether LTB_4 was involved in celecoxib-mediated inhibitory effect on human colon cancer HT-29 cells and human prostate cancer PC-3 cells. Our data showed that survival of both cell lines was obviously suppressed after celecoxib treatment for 72 h in a concentration-dependent manner. However, only in HT-29 cells, this inhibitory effect could be reversed by LTB_4, which promoted survival of HT-29 cells rather than PC-3 cells. Consistent with these results, lioxygenase (LOX) potent inhibitor nordihydroguaiaretic acid (NDGA) had a higher inhibitory effect on HT-29 cells than PC-3 cells. Additionally, ELISA results showed that celecoxib could suppress expression of LTB_4 in both cell lines, whereas, inhibition of PGE_2 was only detected in HT-29 cells. These results indicate that the anticancer effect of celecoxib is COX-2-independent in HT-29 and PC-3 cells and in HT-29 cells primarily via down-regulating LTB_4 production.

1. Introduction

Epidemiological studies provide evidence that a high-fat diet can be associated with an increased risk for cancer, in particular colorectal, prostate and pancreatic cancer [1,2]. Arachidonic acid is one major ingredient of the membranes and via two major metabolic pathways, the cyclooxygenase (COX) pathway and the lipoxygenase (LOX) pathway, metabolized to prostaglandins and leukotrienes, which are represented by prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4), respectively [2]. These biologically active lipids are involved in chronic inflammation and cancer [1–4].

There are two known COX isoforms, COX-1 and COX-2, with distinct expression patterns and biological activities [2]. COX-1 is constitutively expressed in most tissues, whereas, COX-2 is absent from most tissues but is highly induced by pro-inflammatory stimuli and is often actively expressed in various epithelial cancers [3,5]. COX-2-derived prostaglandins, particularly PGE_2, have been associated with antiapoptotic as well as proliferation-promoting effects [2,3]. Since celecoxib is able to inhibit both cell proliferation *in vitro* and tumor growth *in vivo*, at first glance it appears that the pronounced anticancer effects of celecoxib are primarily due to its ability to inhibit COX-2. Other studies, however, have demonstrated that non-COX-2 targets, such as 3-phosphoinositide-dependent protein kinase-1 (PDK-1) [6] and p53 [7], are involved in celecoxib-mediated antiproliferative effects in prostate [8] and colon [9–12] cancer cells *in vitro*.

Until now most of the effort has focused on the role of cyclooxygenase products in carcinogenesis, very little is known about the roles of lipoxygenase products, particularly LTB_4, in this process. However, emerging data suggest that LTB_4 has important roles in the prostate and colon carcinogenesis [4,13,14]. Based on these observations, we evaluated the role of LTB_4 in celecoxib-mediated anticancer effect on colon cancer HT-29 cells and prostate cancer PC-3 cells *in vitro*.

2. Materials and methods

2.1. Materials

RPMI 1640 and F12 media were purchased from Invitrogen (Green Island, NY). Fetal bovine serum (FBS) was from Sijiqing (Hangzhou, Zhejiang, China). PGE_2 and LTB_4 enzyme-linked immunosorbent assay (ELISA) kits were from R&D (Abingdon, UK). Celecoxib was from Pfizer (New York, NY). Nordihydroguaiaretic acid (NDGA) was purchased from Sigma Chemicals (St. Louis, MO). LTB_4 was purchased from Cayman Chemicals (Ann Arbor, MI). For studies, these agents were dissolved in dimethyl sulfoxide (DMSO) and were then added to the cells in medium with the final DMSO concentration kept $\leqslant$0.2%.

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doi:10.1016/j.bbrc.2010.10.022

2.2. Cell lines and cell culture

The human colon cancer HT-29 cells and human prostate cancer PC-3 cells were obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai Institute of Cell Biology, Chinese Academy of Sciences). PC-3 cells were cultured in F12 media, and HT-29 cells in RPMI 1640 media, which were supplemented with 10% FBS plus 100 units/ml penicillin and 100 μg/ml streptomycin. For experiments, cells were fed with fresh medium every third day and digested at a confluence of about 80%.

2.3. Cell Viability

Cells were seeded onto 96-well plates at 5×10^3 cells per well and each group consisted of six parallel wells. After reaching 50% confluence, they were incubated in fresh medium with or without the appropriate treatments. After the required period of culture, cell viability was determined by MTT assay. During the treatment, the percentage of cells floating in the medium increased over time. Both adherent and floating cells were collected for the assessment.

2.4. Measurement of PGE_2 and LTB_4 levels

Levels of PGE_2 and LTB_4 released in media were measured using PGE_2 and LTB_4 ELISA kits, respectively. Medium was sampled, centrifuged to remove floating cells and frozen immediately at −70 °C until assay. The assay was performed according to the manufacturer's instructions, following dilution to ensure that readings were within the limits of accurate detection by the assay.

2.5. Statistical analysis

All quantitative variables are presented as means ± SD (standard deviation). The difference between two groups was assessed with the use of an independent *t*-test. We compared the differences of three or more groups with a oneway ANOVA. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Celecoxib induces cell death of HT-29 and PC-3 cells

Celecoxib with various concentrations was used to treat HT-29 and PC-3 cells for 72 h. Under the phase contrast microscope, the cells exhibited a dramatic morphologic change as well as inhibition of cell survival after drug treatment. The cell viability in response to celecoxib treatment was assayed by MTT assay. As shown in Fig. 1, significant induction of cell death was observed in both cell lines in a dose-dependent manner, whereas, this effect was obviously higher in HT-29 cells (*IC50* = 40 μM) than in PC-3 cells (*IC50* = 70 μM) under concentrations less than 80 μM.

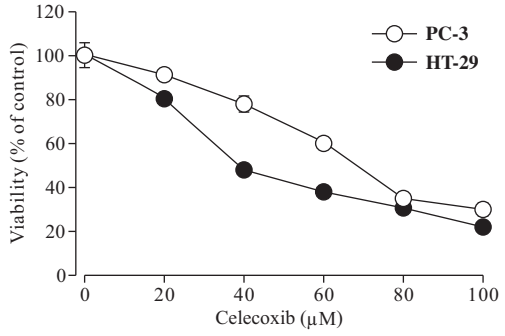

Fig. 1. Inhibitory effect of celecoxib on cell viability. HT-29 and PC-3 cells were treated with celecoxib at increasing concentrations and harvested 72 h later for MTT assay, as described in Section 2. Values are average of duplicates, standard deviation (SD) were within 10%.

3.2. Effect of celecoxib on production of PGE_2 and LTB_4

Amounts of PGE_2 and LTB_4 in medium were evaluated after celecoxib treatment for 72 h. All doses of celecoxib significantly reduced LTB_4 secretion in both cell lines (Fig. 2A and B), indicating that celecoxib is a potent inhibitor of 5-LOX, which induces LTB_4 production. In contrast with LTB_4, the influence of celecoxib on PGE_2 was different in HT-29 and PC-3 cells. As shown in Fig. 2, celecoxib treatment obviously inhibited PGE_2 production in HT-29 cells (Fig. 2A), however, the level of PGE_2 was not changed in PC-3 cells after celecoxib treatment (Fig. 2B).

3.3. Exogenous LTB_4 only reverses celecoxib-mediated inhibitory effect in HT-29 cells

Because celecoxib caused cell inhibition in both cell lines and inhibited LTB_4 secretion, we hypothesized that this inhibitory effect was LTB_4 dependent. To determine whether celecoxib-mediated inhibitory effect could be reversed by exogenous LTB_4, LTB_4 was added to cultures of HT-29 and PC-3 cells treated with constant dose (80 μM) of celecoxib. Varying amounts of LTB_4 (0.001–10 nM) were added to the medium in order to take into account the fact that some of the LTB_4 may degrade or be internalized into cells. In PC-3 cells, cell inhibition induced by 80 μM celecoxib could not be prevented by exogenous LTB_4 (Fig. 3B), suggesting that celecoxib-induced anticancer effect in PC-3 cells may be independent of LTB_4 level. However, addition of 10 nM LTB_4 almost reversed the inhibitory effect induced by 80 μM celecoxib in HT-29 cells (Fig. 3A), suggesting that celecoxib-mediated anticancer effect in this cell line may be dependent on the level of LTB_4.

3.4. Effects of LTB_4 and NDGA on viability of HT-29 and PC-3 cells

In order to confirm the results depicted above, we investigated the role of exogenous LTB_4 alone in HT-29 and PC-3 cells. As shown in Fig. 4, LTB_4 promoted the viability of HT-29 cells, but inhibited that of PC-3 cells (Fig. 4A and B, respectively). However, these effects of LTB_4 on cancer cell survival were mild in our study.

As LTB_4 is a critical component of 5-LOX pathway, we also explored the importance of LOX pathway in survival of both cell lines by using a potent LOX inhibitor NDGA. Results showed that NDGA obviously inhibited the viability of HT-29 cells in a dose-dependent manner, whereas, NDGA inhibited that of PC-3 cells in a limited extent.

4. Discussion

Celecoxib is a selective COX-2 inhibitor possessing anticancer effects for many cancers. Although various COX-2-independent chemo-preventive and tumor-regressive activity have been suggested, the respective non-COX-2 targets of celecoxib are still a matter of research. The results presented here clearly show that down-regulation of LTB_4 synthesis is the primary mechanism of celecoxib-mediated anticancer effect in human colon cancer HT-29 cells.

Several investigators have suggested that celecoxib has anticancer activity via COX-2-dependent and COX-2-independent pathways. It is reported that inhibition of PGE_2 synthesis is an early, but not sufficient, step in the mechanism of celecoxib-mediated cell growth inhibition [12]. As shown in our work, celecoxib suppressed the survival of both COX-2-positive HT-29 and COX-2-deficient PC-3 cells, whereas, celecoxib had a higher inhibitory