Effects of Cyclooxygenase and Lipoxygenase Inhibitors on the Proliferation of Colon Cancer Cells and Their Production of Eicosanoids

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Purpose: Epidemiological and laboratory studies suggest that nonsteroidal antiinflammatory drugs (NSAIDs) reduce the risk of colon cancer and that the inhibition of colon cancer is mediated through modulation of eicosanoid production. The present study examined the effect of cyclooxygenase (COX) and lipoxygenase (LOX) inhibitors on colon cancer cell growth and prostaglandin E₂ (PGE₂) or leukotriene B₄ (LTB₄) secretion by these cells.

Materials and Methods: The human colon adenocarcinoma cell lines, Caco-2 and HT-29 cells, were cultured in serum-free medium with various concentrations of indomethacin, piroxicam or esculetin in the presence of 0.15 μ M or 10 μ M linoleic acid. Cell number was estimated by MIT assay and PGE₂ and LTB₄ were analyzed by enzyme immunoassay.

Results: The NSAIDs inhibited cell proliferation in a concentration-dependent manner. However, the potency and efficacy of each drug varied in the two cell lines. In Caco-2 cells, the effect of esculetin was higher than that of indomethacin, and piroxicam had no effect. In HT-29 cells, only indomethacin significantly inhibited cell proliferation. All three agents inhibited PGE₂ secretion in

a dose-dependent manner; the effect of indomethacin was highest and that of esculetin lowest. The secretion of LTB₄ was increased by indomethacin and piroxicam but decreased by esculetin. The effects of these drugs on cell proliferation and eicosanoid secretion were not influenced by linoleic acid concentrations in the culture media. Neither exogenous PGE₂ nor LTB₄ affected cell proliferation. The results of Pearson correlation analyses revealed that changes in cell proliferation were somewhat related to both concentrations of NSAIDs in the culture medium and production of PGE₂ and LTB₄.

Conclusion: The present data suggests that the anti-proliferative effect of NSAIDs may not be entirely attributed to changes in the production of PGE₂ and/or LTB₄ in the two colon cancer cell lines. These NSAIDs may inhibit cell proliferation largely independent of their ability to modulate eicosanoid synthesis. (*Cancer Research and Treatment* 2001;33:404-413)

Key Words : Colon neoplasm, Cyclooxygenase inhibitor, Lipoxygenase inhibitor, Eicosanoid

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가 (4,5). NSAIDs가
NSAIDs가
가 . NSAIDs가 cyclooxygenase (COX)
lipoxygenase (LOX)
eicosanoid 가
. Eicosanoid , , (6,7)
COX PGs eicosanoid
COX-1 COX-2
가 가 . COX-1 COX-2
PG
. COX-1
, COX-2
가 가
(6,7), (8)
COX-2 , ,
가 (9,10).
COX-2
(11),
(12). PGE₂ COX-2 bcl-2
apoptosis , COX-2
apoptosis (12,13).
LOX leukotriene (LT) hydroxy-
eicosanoic acid (HETE) . Bortuzzo
(14) HT-29 HCT-15 LTB₄
12(R)-HETE 가 가
5-LOX BWA4C BWB70C
10 μM 가
NSAIDs COX
LOX apoptosis 가
가
COX LOX 가 PGE₂
LTB₄
eicosanoid .

1)

Caco-2, HT-29
cell American Type Culture Collection (Rockville, MD)
Dulbecco's modified

Eagle's medium/Nutrient Mixture Ham's F12 (DMEM/F12)
fetal bovine serum (FBS), trypsin-EDTA, penicillin-streptomycin, transferrin, selenium Gibco-BRL (Gaithersburg, MD)
. Linoleic acid, indomethacin, piroxicam, esculetin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), bovine serum albumin (BSA)
Sigma Chemical Co. (St. Louis, MO)

1

Corning (Corning, NY)

2)

37°C, CO₂ incubator (5% CO₂/95% air)
DMEM/F12

DMEM/F12 10% FBS 가

(penicillin 100 units/ml, streptomycin 100 μg/ml) 가

가 80% dish

PBS monolayer 0.25% trypsin-2.65 mM

EDTA 2

3) COX LOX

COX LOX

10% FBS가

HT-29 5 × 10⁴ cells/well,

Caco-2 4 × 10⁴ cells/well 가 24 well plate

. 24 serum-free medium

(SFM) 24 serum starvation

. SFM FBS가 DMEM/F12 transferrin (5 μg/

ml), selenium (5 g/ml), penicillin (100 units/ml), streptomycin

(100 μg/ml) 가 . SFM

0.15 μM ,

10 μM 가 가

BSA

linoleic acid-BSA

BSA 4 : 1 .

COX LOX 0, 25, 50, 100 μM

가 2 , 4 , 6 MTT

(15) . MTT

가 MTT

formazan

formazan isopropanol 570

nm

MTT assay (r=0.99) COX indomethacin, piroxicam, LOX esculetin DMSO 100 mM stock solution -20°C well DMSO

4) PGE₂ LTB₄ eicosanoid serum-free conditioned media glacial acetic acid 가 pH 4.0 (3 : 1, v/v) PG LT speed vac eicosanoid enzyme immunoassay kit (Cayman Chemicals Co, Ann Arbor, MI)

5) PGE₂ LTB₄ Eicosanoid Caco-2 10% FBS가 4 × 10⁴ cells/well 24 well plate 24

serum-starvation 0 100 μM 가 LTB₄ 0 1 μM MTT assay 24, 48, 72

6) SAS ANOVA Duncan's multiple test =0.05 COX LOX , PGE₂, LTB₄ Pearson

1) COX LOX 가

LOX 가 COX

10 μM serum-free 가 COX (indomethacin, piroxicam) LOX (esculetin) 0, 25, 50, 100 μM 가

MTT

Fig. 1. Effect of COX and LOX inhibitors on the proliferation of Caco-2 cells in the presence of 10 μM LA. Cells were plated in 24-well plates in DMEM/F12 supplemented with 10% fetal bovine serum. One day later, the monolayers were serum-starved with serum-free DMEM/F12 supplemented with 5 μg/ml transferrin and 5 ng/ml Se for 24 hours. After serum starvation (0 day), cells were incubated in serum-free medium containing 10 μM LA with or without various concentrations of COX or LOX inhibitors. Cell numbers were estimated by the MTT assay. The data were expressed as a percentage of day 0 value. Each bar represents the mean ± SEM (n=6). ANOVA, followed Duncan's multiple range test at each time point was used to determine significant differences among the treatment groups. Comparisons between groups that yielded significant differences (p < 0.05) are indicated by different letters above each bar. Data for groups that share the same letter are not significantly different.

Fig. 2. Effect of COX or LOX inhibitor on the proliferation of HT-29 cells in the presence of 10 μM LA. Cells were plated and cultured as described in Fig. 1. After serum starvation (0 day), cells were incubated in serum-free medium containing 10 μM LA with or without various concentrations of COX or LOX inhibitors. Cell numbers were estimated by the MTT assay. The data were expressed as a percentage of day 0 value. Each bar represents the mean ± SEM (n=6). ANOVA, followed Duncan's multiple range test at each time point was used to determine significant differences among the treatment groups. Comparisons between groups that yielded significant differences ($p < 0.05$) are indicated by different letters above each bar. Data for groups that share the same letter are not significantly different.

Fig. 3. Effect of cyclooxygenase (COX) and lipoxygenase (LOX) inhibitors on the proliferation of Caco-2 cells in the presence of 0.15 μ M LA. Cells were plated and serum- starved as described in Fig. 1. After serum starvation, cells were incubated in serum-free medium with or without various concentrations of COX or LOX inhibitors. Cell numbers were estimated by the MTT assay. The data were expressed as a percentage of day 0 value. Each bar represents the mean $\pm$ SEM (n=6). ANOVA, followed Duncan's multiple range test at each time point was used to determine significant differences among the treatment groups. Comparisons between groups that yielded significant differences ($p < 0.05$) are indicated by different letters above each bar. Data for groups that share the same letter are not significantly different.

Fig. 4. Effect of COX and LOX inhibitors on PGE₂ and LTB₄ concentrations in the conditioned medium by Caco-2 cells in the presence of 10 μ M LA. Cells were plated and cultured as described in Fig. 1. After serum starvation, cells were incubated in serum-free medium containing 10 μ M LA with or without various concentrations COX or LOX inhibitors. Conditioned media were acidified with glacial acetic acid, and PGE₂ or LTB₄ was extracted with ethyl acetate and estimated by a competitive enzyme immunoassay. ANOVA, followed Duncan's multiple range test with each inhibitor was used to determine significant differences among the treatment groups. Comparisons between groups that yielded significant differences ($p < 0.05$) are indicated by different letters above each bar. Data for groups that share the same letter are not significantly different.

dent . LTB₄ esouletin 10 μ M (Fig. 4).
 100 μ M 가 . Indomethacin 50 NSAID
 μ M LTB₄ 가 piroxicam Caco-2 cell PGE₂ LTB₄
 25 μ M LTB₄ 가 가 가 10 μ M (Fig. 6).
 가 가 LTB₄ 가
 (Fig. 5). 가 0.15 μ M 3) PGE₂ LTB₄
 Caco-2 cell PGE₂ (Fig. 6) Caco-2 indomethacin, piroxicam, esouletin

Fig. 5. Effect of COX and LOX inhibitors on PGE₂ and LTB₄ concentrations in the conditioned medium by HT-29 cells in the presence of 10 μM LA. Cells were plated and cultured as described in Fig. 1. After serum starvation, cells were incubated in serum-free medium containing 10 μM LA with or without various concentrations COX or LOX inhibitors. Conditioned media were acidified with glacial acetic acid, and PGE₂ and LTB₄ was extracted with ethyl acetate and estimated by a competitive enzyme immunoassay. ANOVA, followed Duncan's multiple range test with each inhibitor was used to determine significant differences among the treatment groups. Comparisons between groups that yielded significant differences ($p < 0.05$) are indicated by different letters above each bar. Data for groups that share the same letter are not significantly different.

Fig. 6. Effect of COX and LOX inhibitors on PGE₂ and LTB₄ concentrations in the conditioned medium by Caco-2 cells in the presence of 0.15 μM LA. Cells were plated and cultured as described in Fig. 3. Conditioned media were collected and acidified with glacial acetic acid. PGE₂ and LTB₄ was extracted with ethyl acetate and estimated by a competitive enzyme immunoassay. ANOVA, followed Duncan's multiple range test with each inhibitor was used to determine significant differences among the treatment groups. Comparisons between groups that yielded significant differences ($p < 0.05$) are indicated by different letters above each bar. Data for groups that share the same letter are not significantly different.

가	PGE ₂	LTB ₄	0.001, $p < 0.01$	. Indome-
Table 1	. Caco-2	가 PGE ₂	가 LTB ₄	($p <$
indomethacin, piroxicam, esculetin	가	. Piroxicam	가	PGE ₂
($p < 0.001$).	Indomethacin	가	가	, esculetin
PGE ₂	($p < 0.001$)	가	PGE ₂	LTB ₄
, LTB ₄	($p < 0.001$)	($p < 0.001$)	.	
. Piroxicam	가	PGE ₂	LTB ₄	p
< 0.01	. Esculetin	가	Table 2	HT-29
PGE ₂	LTB ₄	p <	indomethacin, piroxi-	
			cam, esculetin	가
			PGE ₂	LTB ₄
			. HT-29	

Table 1. Correlation between the concentrations of individual COX and LOX inhibitors, cell growth, PGE₂, and LTB₄ concentrations in Caco-2 cells by Pearson correlation analysis

	Indomethacin	Cell growth	PGE ₂	LTB ₄
Indomethacin	1.0000			
Cell growth	−0.9795 [‡]	1.0000		
PGE ₂	−0.6980 [‡]	0.6941 [†]	1.0000	
LTB ₄	0.9872 [‡]	−0.9480 [‡]	−0.6423 [‡]	1.0000
	Piroxicam	Cell growth	PGE ₂	LTB ₄
Piroxicam	1.0000			
Cell growth	−0.7858 [‡]	1.0000		
PGE ₂	−0.8213 [‡]	0.5418 [†]	1.0000	
LTB ₄	−0.5655 [‡]	0.5359 [†]	0.1164	1.0000
	Esculetin	Cell growth	PGE ₂	LTB ₄
Esculetin	1.0000			
Cell growth	−0.9534 [‡]	1.0000		
PGE ₂	−0.8610 [‡]	0.8830 [‡]	1.0000	
LTB ₄	−0.6313 [‡]	0.5303 [†]	0.6804 [‡]	1.0000

*p < 0.05, [†] p < 0.01, [‡] p < 0.001**Table 2.** Correlation between the concentration of individual COX and LOX inhibitors, cell growth, PGE₂, and LTB₄ concentrations in HT-29 cells by Pearson correlation analysis

	Indomethacin	Cell growth	PGE ₂	LTB ₄
Indomethacin	1.0000			
Cell growth	−0.8919 [‡]	1.0000		
PGE ₂	−0.8733 [‡]	0.7385 [†]	1.0000	
LTB ₄	0.9061 [†]	−0.9453 [‡]	−0.8301 [‡]	1.0000
	Piroxicam	Cell growth	PGE ₂	LTB ₄
Piroxicam	1.0000			
Cell growth	−0.74431*	1.0000		
PGE ₂	−0.8800 [‡]	0.3254	1.0000	
LTB ₄	0.4225*	0.1004	−0.5438 [†]	1.0000
	Esculetin	Cell growth	PGE ₂	LTB ₄
Esculetin	1.0000			
Cell growth	−0.8213 [‡]	1.0000		
PGE ₂	−0.4550*	0.4741*	1.0000	
LTB ₄	−0.7151 [‡]	0.5563 [†]	0.4320*	1.0000

*p < 0.05, [†] p < 0.01, [‡] p < 0.001

Fig. 7. Effect of exogenous PGE₂ (A) and LTB₄ (B) on the proliferation of Caco-2 cells. Cells were plated and serum-starved described in Fig. 1. After serum starvation, cells were incubated in the absence or presence of various concentrations of exogenous PGE₂ or LTB₄. Cell numbers were estimated by the MTT assay. Each bar represents the mean $\pm$ SEM (n=8). Comparisons between different concentrations of individual eicosanoids that yielded significant differences ($p < 0.05$) are indicated by different letters above each bar.

indomethacin, PGE₂, LTB₄

Caco-2, piroxicam, Caco-2

piroxicam, LTB₄

($p < 0.05$)

PGE₂, LTB₄

가, Esculetin

Caco-2, 가

, PGE₂, LTB₄

($p < 0.001$, $p < 0.05$, $p < 0.001$)

NSAIDs가 COX, LOX

, NSAIDs가

eicosanoid

COX, indomethacin

piroxicam, LOX, esculetin, 가, PGE₂, LTB₄

가, COX

4) PGE₂, LTB₄가

Eicosanoid, 가, 가

eicosanoid, Caco-2, 가

, PGE₂

0, 100 μM, 가, 24, 48, 72

100 μM

PGE₂, 가

, 0, 1 μM, LTB₄

가, PGE₂, 가

(Fig. 7).

NSAIDs (4-6) NSAIDs가

가

NSAIDs가 COX, LOX

, NSAIDs가

eicosanoid

가, Eicosanoid

,

PGE₂, 가

7.5 mg piroxicam

PGE₂, 가

(16).

in vitro

COX, COX-2

가, COX, LOX

(17), (18)

(AGS)

5-LOX

. LOX, nordihydroguaiaretic acid (NDGA)

NCI-H1264, 5-LOX

. LOX, 가

(19).

- NDGA가 apoptosis
LOX
(18).
COX LOX
PGE₂ LTB₄
,
COX -indomethacin, piroxicam indomethacin
가 . Piroxicam 100 μ M
Caco-2 가
HT-29
. LOX esculetin COX
가 가 COX
LOX COX Noguchi (20)
가 , (MDA-MB-
231) piroxicam esculetin LOX COX
, LOX
Hofmanova (21) HS578T
monoblastoid U937 , piroxicam
COX
piroxicam
PGE₂ piroxicam 가
가 PGE₂
Table 1, 2
COX LOX
PGE₂ LTB₄
가 COX
cosanoid 가
LTB₄ 가
PGE₂ 가
가 (Fig. 7) eicosanoid
가 . Parker (22) HT-29 PGE₂
가 10 μ M 가
가 100 μ M 가
eicosanoid
가 NSAID
eicosanoid
Caco-2 HT-29
COX LOX
. COX LOX
LTB₄ 가
LOX eicosanoid 가 COX
PGE₂ LTB₄
PGE₂ LTB₄ 가
PGE₂ LTB₄
가 ,
COX LOX eicosanoid
가 .
가 .

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