



Acute changes in colonic PGE₂ levels as a biomarker of efficacy after treatment of the Pirc (F344/NTac-Apc^{am1137}) rat with celecoxib

Changhong Yun^{1,2} · Wan-Mohaiza Dashwood³ · Li Li¹ · Taijun Yin¹ · Ahmet M. Ulusan³ · Katherine Shatzer¹ · Song Gao¹ · Ke-He Ruan¹ · Ming Hu¹

Received: 20 August 2019 / Revised: 19 September 2019 / Accepted: 26 September 2019 / Published online: 3 December 2019
© Springer Nature Switzerland AG 2019

Abstract

Objective This study sought to evaluate short-term treatment with COX-2 inhibitors and acute changes in colonic PGE₂ levels as predictors of long-term efficacy in a genetic model of colorectal cancer.

Methods Celecoxib oral suspension (40 mg/kg BID) was dosed to Apc-mutant Pirc (F344/NTac-Apc^{am1137}) rats for 4 days (short-term group), or the equivalent dose of 1500 ppm celecoxib was administered in the diet for 4 months (long-term group). Percent inhibition of colonic PGE₂ was calculated, and the reduction in colonic PGE₂ was assessed in relation to suppression of adenomatous colon polyps.

Results Colonic mucosa PGE₂ was fourfold higher in Pirc than in F344 wild-type rats (21 vs. 5.6 pg/mg epithelial tissue), due at least in part to higher COX-2 expression, and this was confirmed by elevated PGE₂-d11 levels in Pirc colonic S9 incubations. In the 4-day study, dose-dependent reductions in PGE₂ were observed in colonic epithelium (-33% (P>0.05) and -57% (P=0.0012)), after low- and high-dose celecoxib treatments of 4 mg/kg and 40 mg/kg (bid), respectively. In the 4-month study, 1500 ppm celecoxib suppressed colonic epithelium PGE₂ by 43.5%, and tumor multiplicity by 80% (P<0.0015). Suppression of plasma 6-keto PGF_{1α} also was corroborated following long-term treatment with 1500 ppm celecoxib (P<0.05).

Conclusions Acute changes in colonic mucosa PGE₂ provided a rapid means of predicting long-term chemopreventive effects from celecoxib, and might be useful for screening of new COX-2 inhibitor compounds.

Keywords Pirc rat · FAP · Prostaglandin E₂ · Chemoprevention · COX-2 inhibitor · Celecoxib

Abbreviation

AA	Arachidonic acid
APC	Adenomatous polyposis coli
COX	Cyclo-oxygenase
Coxib	COX-2 inhibitor
CRC	Colorectal cancer
FAP	Familial adenomatous polyposis

FDA	Food and Drug Administration
Pirc rat	Polyposis in the rat colon rat (F344/NTac-Apc ^{am1137})
IC ₅₀	Half-maximal inhibitory concentration
LC-MS	Liquid chromatography–mass spectrometry
NSAID	Non-steroidal anti-inflammatory drug
PGE ₂	Prostaglandin E ₂
6-keto PGF _{1α}	6-Keto prostaglandin F _{1α}

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00011-019-01300-5>) contains supplementary material, which is available to authorized users.

✉ Ming Hu
mhu@uh.edu

¹ Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, TX 77204, United States

² Present Address: Safety Assessment and Laboratory Animal Resources, Merck & Co., West Point, PA 19486, USA

³ Center for Epigenetics and Disease Prevention, Texas A&M University, Houston, TX 77030, USA

Introduction

Familial adenomatous polyposis (FAP) is a rare inherited disease that results from an autosomal dominant genetic alteration in the adenomatous polyposis coli (APC) gene [1]. Patients with this disease [2] characteristically develop hundreds to thousands of adenomatous polyps (adenomas) in the duodenum, colon, and rectum beginning in adolescence. Left untreated, patients with FAP have almost 100%

morbidity and mortality from colorectal cancer (CRC) [3]. Colonoscopy in the clinical setting can enhance survival, via early detection, but this preemptive procedure often has low patient compliance. In subjects with FAP, colonoscopy repeated every 2–3 years also typically fails to suppress long-term polyp recurrence. Colectomy with ileorectal or ileoanal anastomosis is recommended, but organ removal can severely impact quality of life. Beyond surgical removal of adenomatous precursor lesions, there is continued interest in chemoprevention strategies that might benefit FAP patients [4].

One potential pharmacological target in adenomatous tissues of the GI tract is the cyclooxygenase-2 (COX-2) enzyme [5]. The COX-2 expression increases markedly in the majority of colorectal adenomas and adenocarcinomas, compared to low levels in healthy colonic mucosa. Prostaglandin E₂ (PGE₂), an oncogenic COX-2-derived metabolite, plays an important role in maintaining tumor integrity and metastasis [6], also promoting COX-2 expression via a positive feedback loop [7, 8].

Animal models mirroring human CRC can assist in developing new chemopreventive therapies for FAP patients [9]. Carcinogen-induced models using azoxymethane (AOM), 1,2-dimethylhydrazine (DMH), or 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) have been reported [10], with caveats including long latency, low tumor multiplicity, and incomplete penetrance. Mouse models [11] with defects in the *Apc* gene have been used for the development and testing of chemopreventive agents, but typically fail to recapitulate the characteristics of human FAP [12]. Thus, tumors predominantly arise in the small intestine, rather than the colon, and the short lifespan often precludes extensive prevention studies. However, the *Apc*-mutant polyposis in rat colon (Pirc) model [13] offers advantages, including the high number of polyps arising in the colon, and a lifespan suitable for primary and secondary preventions studies [14–17]. The big body size in rats also facilitates additional tissue analyses, such as plasma biomarkers potentially linked to disease outcomes. These and other observations suggest that the Pirc model is a useful mimic of human FAP, and valuable for investigating chemoprevention strategies [14, 18]. Previous studies typically were conducted over several months in the Pirc model, but we sought to evaluate short-term biomarkers altered within 1 week that might predict efficacy from long-term treatments, starting with COX-2 inhibition as proof-of-principle.

In the present study, we found that PGE₂ levels were much higher in Pirc colonic mucosa and adenomatous polyps compared to the levels in wild-type rats. This finding in the rat was also corroborated by an *in vitro* colonic S9 incubation assay with deuterated labeled substrate arachidonic acid-d11 (AA-d11). High-dose celecoxib remarkably suppressed colonic mucosa PGE₂ and tumor multiplicity

after 4 months treatment, and the 4-day colonic PGE₂ level reflected that observed after 4 months of treatment. Thus, short-term PGE₂ inhibition *in vivo* might serve as a useful predictive biomarker for long-term efficacy screening of other COX-2 inhibitors with enhanced therapeutic outcomes.

Materials and methods

Materials and reagents

PGE₂, PGE₂-d4, PGE₂-d9, arachidonic acid-d11, 6-keto PGF_{1 α} , 6-keto PGF_{1 α} -d4, celecoxib, and rofecoxib purchased from Cayman Chemical Inc. (Ann Arbor, MI). Ethyl acetate, K₂HPO₄, and KH₂PO₄ were bought from VWR Corporate (Radnor, PA). Ammonium hydroxide and sucrose were supplied by Sigma-Aldrich (St. Louis, MO); and EDTA-Na₂·6H₂O by J.T. Baker (Phillipsburg, NJ). Acetonitrile (HPLC grade) was obtained from OmniSolv (Billerica, MA). Distilled water, prepared from demineralized water, was used throughout the study. C18 SPE cartridge (Bond Elut Plexa, 60 mg, 3 ml) is the purchased from Agilent Technologies.

Animal treatments and sample collections

Adult Pirc rats originated from McArdle Laboratory for Cancer Research (University of Wisconsin–Madison) were housed in Center for Epigenetics and Disease Prevention (Houston, TX). Wild-type control F344 rats (male and female) were purchased from Charles River Laboratories and were killed at the age of 13 months. All the experimental protocols were approved by Institutional Animal Care and Use Committee (IACUC), Institute of Biosciences and Technology, Texas A&M University.

Short-term celecoxib dosing study Celecoxib oral suspensions (4 or 40 mg/kg, dissolved in 0.5% methyl cellulose and 0.1% Tween-80) were given to the female Pirc rats (aged 13 months) twice daily for a continuous 4 days (12-h dosing interval, and eight doses in total). Same blank vehicle was given to the wild-type and Pirc controls. Whole blood samples were collected from the tail vein on 0, 1, 2, 3, 4 and 6 h after the last dose on Day 4. Approximately 3 ml of plasma was immediately separated from the whole blood drawn from cardiac puncture before scarification by a low-speed centrifuge (1200 RPM * 8 min at 4 °C). The Pirc body was cut from abdomen to anus, and the entire colon was harvested. After removal of the polyps, the whole mucosa was scrapped in fresh. The visible polyps were counted with measurement of their sizes by using calipers and then sorted based on their sizes: small (size < 1 mm), medium (1 mm < size < 2 mm) and large (size > 2 mm). The volume was calculated by using the equation $V = 4/3 \pi * (\text{length}/2)$

* (width/2) * (height/2). All the samples were then kept in the -80°C freezer until further analysis.

Long-term adenoma prevention study 40 six-week-old male Pirc rats maintained in standard cages were divided into two groups feeding with either AIN93G control diet (Research diet, NJ) or the diet containing 1500 ppm celecoxib (approximately equal to 80 mg/kg per day, detailed conversion refer to the supplement) for 4 months. The daily diet consumption was about 12–20 g per rat varied from ages. Rats received the colonic endoscopy on week 10 after initialization of the feeding experiment, to examine the intermediate efficacy. The polyp number and burden grades (Supplementary Figure S2) reading from camera video were recorded [19]. The colon samples were longitudinally opened and flatted on the filter paper after necropsy at the last day. At the end of the study, collection of polyps and mucosa was same as the operation in the short-term described above.

LC–MS methods

The UPLC–MS/MS instruments consisted of a Waters ACQUITY UPLC System (a quaternary solvent manager, a column manager, and a sample manager; Waters Corporation, MA, USA) and an API 5500 Q-Trap (AB Sciex, MA,

USA) with an ESI interface. Data were processed with Analyst software (version 1.52).

All the prostaglandin assays [20] were conducted on a UPLC BEH C18 column (2.1×100 mm, $1.7 \mu\text{m}$, Waters). A binary gradient consisting of 0.1% ammonium hydroxide solution (A) and acetonitrile (B) eluted in program (Table 1), with a flow rate of 0.3 ml/min at 25°C . Celecoxib was analyzed by an Atlantis dC18 column (2.1×50 mm, $3 \mu\text{m}$) with isocratic elution (water/acetonitrile: 55/45, v/v) in 0.5 ml/min at 25°C . Mass spectrometer run in ESI (-) mode with following the parameters: source temperature 600°C ; Curtain Gas 30 psi; Gas1 40 psi; and Gas2 30 psi. Table 2 summarizes all the MRM transitions for all the compounds.

Sample preparations

Colon sample preparation A chilled 10 mM phosphate buffer solution (pH = 7.4) in 600 ml with 51.344 g sucrose and 0.224 g EDTA dihydrate was prepared. The colonic mucosa and polyps were homogenized with 10 ml buffer after measuring the sample weights. 2 ml internalized homogenization samples (internal standards: 50 ng/ml PGE₂-d4 and 100 ng/ml rofecoxib in final concentration) were extracted with 8 ml ethyl acetate (liquid–liquid extraction, LLE), then vortexed and centrifuged. The upper supernatant was collected and transferred to a fresh tube for evaporation using nitrogen stream. The collective dried residual was dissolved in 100 μl reconstitution solvent (acetonitrile: 0.1% ammonia hydroxide = 2:8, v/v), and drawn 5 μl for LC–MS injection.

Plasma sample preparation Systemic PGI₂ was denoted by its stable metabolite 6-keto PGF_{1 α} because PGI₂ is highly unstable (degradation half-time is about 30 s). Plasma sample was purified by the SPE method. Samples were pre-mixed with their specific internal standards (2 ml plasma contained 1.25 ng/ml 6-keto PGF_{1 α} -d4, and 13.3 ng/ml rofecoxib in final concentration). The elution progressed in the following order: 3 ml acetonitrile, 3 ml water, 2 ml internalized plasma, 3 ml water and 1 ml acetonitrile. All the washing eluents were discarded, except the final 1 ml acetonitrile was kept for nitrogen dry. The dried residual was dissolved in 100 μl reconstitution

Table 1 UPLC gradient programs

UPLC Gradient Programs 0.1% ammonium hydroxide solution (A) and acetonitrile (B)					
PGE ₂			6-keto PGF _{1α}		
Time (min)	A %	B %	Time (min)	A %	B %
0.0	87	13	0.0	95	5
6.5	87	13	0.5	88	12
7.5	20	80	5.0	88	12
9.5	20	80	5.5	20	80
10.0	87	13	7.5	20	80
15.0	87	13	8.0	95	5
			10.0	95	5

Table 2 MRM transitions

	MRM (m/z)	DP (V)	CE (V)	CXP (V)	EP (V)
PGE ₂	351.2 \geq 271.2	– 230	– 22	– 11	10
PGE ₂ -d4 (I.S.)	355.3 \geq 275.3	– 230	– 22	– 11	10
PGE ₂ -d9	360.2 \geq 280.2	– 230	– 22	– 11	10
PGE ₂ -d11	362.2 \geq 282.2	– 230	– 22	– 11	10
6-keto PGF _{1α}	369.2 \geq 163.0	– 180	– 34	– 11	10
6-keto PGF _{1α} -d4 (I.S.)	373.3 \geq 166.9	– 180	– 34	– 11	10
Celecoxib	380.3 \geq 316.0	– 220	– 30	– 11	10
Rofecoxib (I.S.)	313.3 \geq 257.1	– 180	– 27	– 11	10

solvent (acetonitrile: 0.1% ammonia hydroxide = 2:8, v/v), and 10 μ l sample for LC–MS injection.

Whole blood sample preparation Aliquot of 20 μ l of the whole blood sample (collected from the tail vein) was diluted with 200 μ l acetonitrile containing 5 μ g/ml rofecoxib, vortex and centrifuge (15,000 RPM). 5 μ l of the supernatant was injected to LC–MS.

In vitro studies

Preparation of colon S9 Colon tissues were harvested from Pirc rat and wild-type F-344 in fresh after scarification. Tissues were minced and then homogenized with the 10 ml buffer at 4 °C with a motor-driven pestle (Pirc polyps and mucosa were combined together, whereas wild type only has mucosa). The homogenate was centrifuged at 10,400g for 15 min at 4 °C. After carefully removal of the fat layer, the S9 supernatant was collected [21]. 1 ml incubation contained 0.15 ml S9 supernatant, 0.84 ml 100 mM KPI solution and 10 μ l 10 mM d11 AA (final organic solvent was 1%). The mixture was placed on 37 °C shaking water bath for 2 h in the presence of sufficient oxygen. In deactivated incubation, colon S9 was deactivated by acetonitrile prior to adding d11 AA.

Biosynthesis and structural confirmation of PGE₂-d11 10 mM stable isotope-labeled arachidonic acid (d11 AA) in final concentration was incubated with about 300 units human recombinant COX-2 enzyme in 1 ml KPI buffer for 3 h. PGE₂-d11 was expected to be obtained after the incubation, as no deuterated hydrogen locates on the active metabolism spot or on the functional group that has H/D exchange. The final product was purified and analyzed using the same method that was applied to colon samples.

Results

Pirc colonic PGE₂

The Pirc “normal” colonic mucosa is PGE₂ about 4 times higher than the wild type (21 pg/mg tissue > 5.6 pg/mg tissue, $P=0.0003$, Fig. 1b). All the polyps were pooled together for the assay, as different sizes of polyps had no difference for PGE₂ background level ($p=0.25$, Fig. 1a). The polyp PGE₂ was about 2.5- to 3.5-fold higher than mucosa PGE₂ ($p<0.001$, Fig. 1a).

The 4-day celecoxib treatment (Fig. 1b) reduced the mucosa PGE₂ by 32.9% (low dose, $p=0.094$) and by 56.7% (high dose, $p=0.0012$) in a dose–response manner. The high-dose celecoxib treatment was deemed to be effective in delaying the tumor progress because the Pirc mucosa PGE₂ (9.1 pg/mg tissue, after treatment) was as low as that of the wild type (5.6 pg/mg tissue). The polyp PGE₂ was also down-regulated by the high-dose celecoxib by 36% (Fig. 1c), although the decline of polyp PGE₂ was not as significant as mucosa PGE₂. The 4-month feeding of 1500 ppm celecoxib (equivalent to 80 mg/kg per day) decreased the mucosa PGE₂ levels by 43.5% ($p=0.0014$) and polyp PGE₂ by 35% ($p=0.13$, Fig. 2).

Efficacy evaluation for celecoxib treatment

The treatment efficacy was observed with endoscopy at 10 weeks and pathological examination at 4 months. The endoscopy revealed 1500 ppm celecoxib reduced the polyp number by sixfold ($P<0.0001$, Fig. 3a). The anatomic endpoint (4 months after treatment, Fig. 3c, d) showed that remarkable regression in polyps number and size with celecoxib feeding (1.8 ± 0.5 polyps/rat with average volume in 23.7 ± 10.8 mm³ vs. 10.1 ± 1.7 polyps/rat with 104.7 ± 25.1 mm³). Notably, 6 Pirc rats (one-third of the

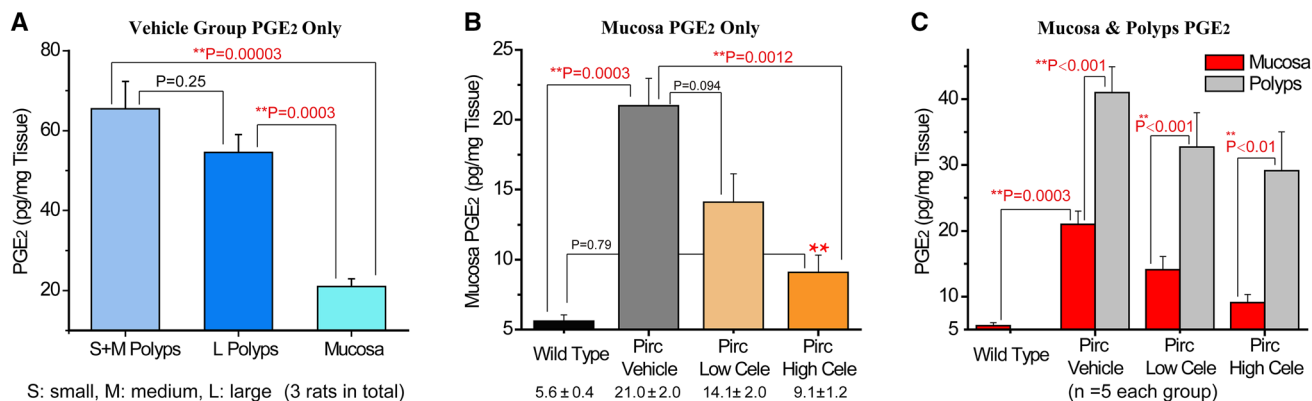


Fig. 1 Colon PGE₂ levels with 4-day celecoxib treatment

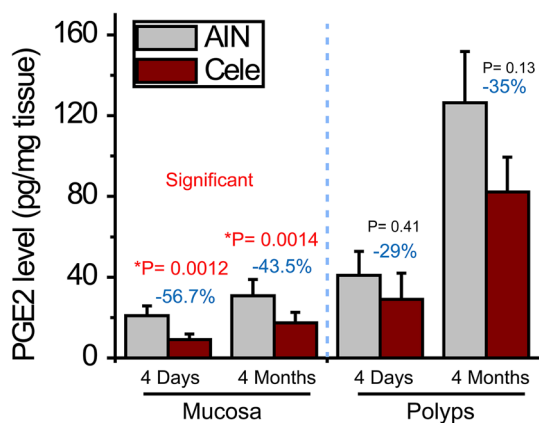


Fig. 2 Colon PGE₂ levels (short-term and long-term celecoxib treatment)

population) dosed with celecoxib had no polyps (example shown in Fig. 4). All the evidence had proven a dose of 1500 ppm celecoxib in the diet was considerably effective against the adenoma.

In vitro colon S9 incubation

The PGE₂-d11 reference standard was biosynthesized in-house (Fig. 5a) as it was commercially unavailable. We confirmed the PGE₂-d11 chemical structure by its deprotonated molecule *m/z* 362.2, fringe-print fragmentation

Fig. 3 Celecoxib long-term feeding outcome. Pirc colonic polyps number and size decreased with treatment

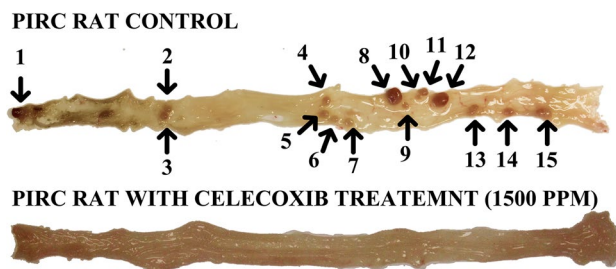
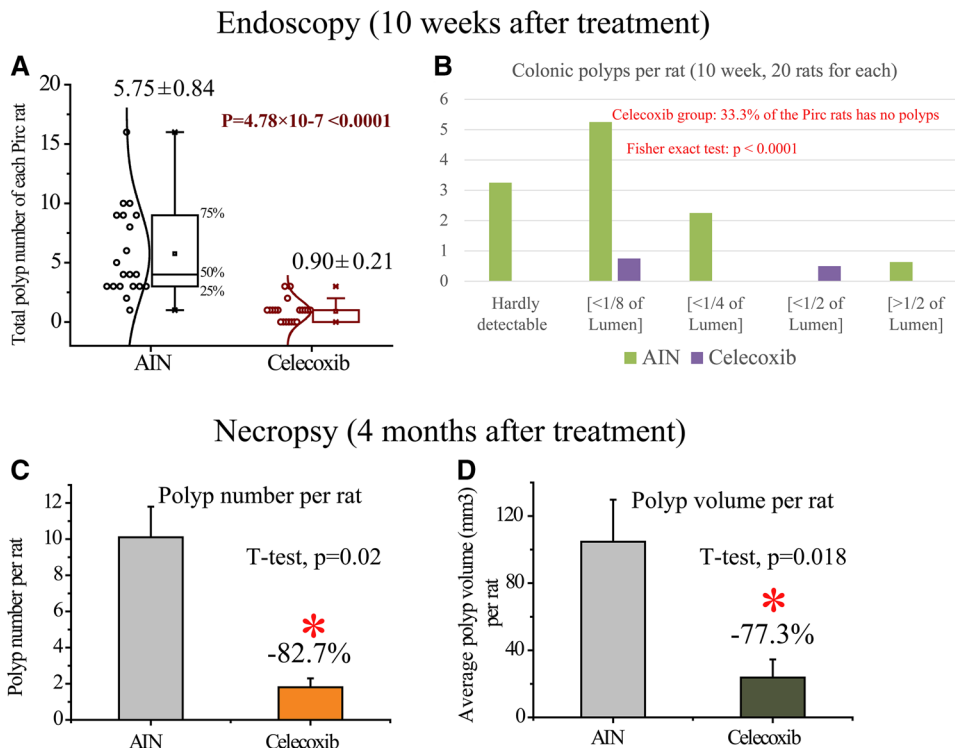


Fig. 4 Comparison of the Pirc colon with and without celecoxib treatment (4-month duration, 1500 ppm celecoxib)

(Supplementary Figure S3), and the identical chromatographic retention that same as PGE₂-d4 (Fig. 5c).

The high upregulation of COX-2 enzyme in Pirc colon tissue was confirmed because the Pirc colon S9 converted AA-d11 to PGE₂-d11 (Fig. 5d). The deactivated incubation was to eliminate the possible interferences originating either from AA-d11 degradation or other endogenous substances. It turned out the metabolite identification did not suffer from undue interference, since there was no peak eluted at 4.4 min (Fig. 5e).

Assessments of CV toxicity-related biomarkers

Plasma 6-keto PGF_{1α} is the biomarker of systemic prostacyclin biosynthesis that related to the cardiovascular (CV) safety (for review, see Ref. [22–25]). Downregulation of

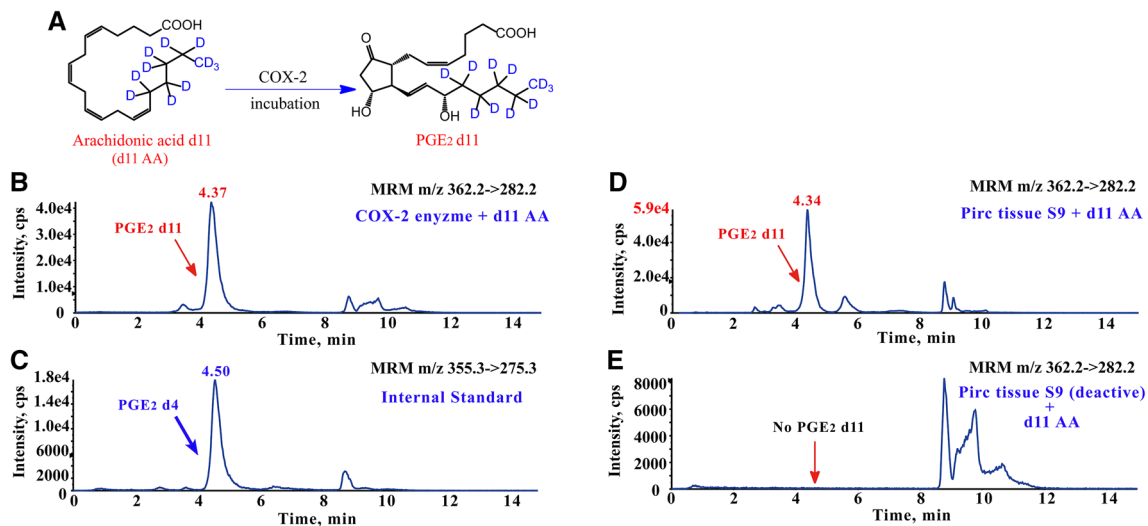


Fig. 5 Confirmation of Pirc colon tissue containing abnormally high level of COX-2 by in vitro S9 incubation

plasma 6-keto $\text{PGF}_{1\alpha}$ is considered as a strong signal of CV toxicity resulting from COX-2 inhibition. The baseline levels of 6-keto $\text{PGF}_{1\alpha}$ was 91.3 ± 13.6 pg/ml, and it decreased by about 70% with 4-day high-dose celecoxib treatment ($P=0.003$, Fig. 6a). The similar decrement was also observed in the 4-month celecoxib feeding ($P=0.006$, Fig. 6b).

Discussion

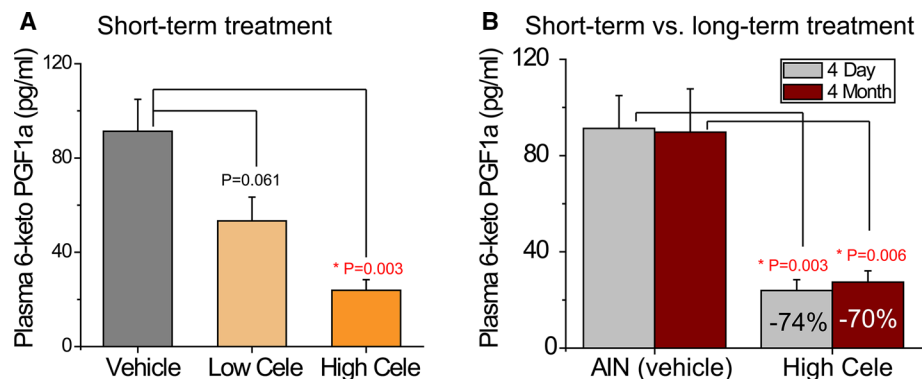
In the present research, we studied the colonic PGE_2 change with 4-day and 4-month celecoxib treatments. The PGE_2 background was much higher than that of wild type, which is consistent with the current concept that PGE_2 promotes colorectal cancer. The in vitro S9 data verified the presence of overexpressed COX-2 enzymes in the Pirc colon tissue and confirmed the feasibility of using acute PGE_2 decrement to predict the outcome of colorectal cancer prevention.

The mucosa PGE_2 level could be a more sensitive biomarker than polyp PGE_2 as it significantly decreased with celecoxib treatment. Remarkable tumor regression was observed in Pirc feeding with celecoxib for 4 months, and meanwhile the mucosa PGE_2 was also found to down-regulate by 44%. Because of the similar decrement of mucosa PGE_2 with short-term and long-term treatment, acute changes in colonic mucosa PGE_2 provided a rapid means of predicting long-term chemopreventive effects from celecoxib.

The acute and long-term celecoxib treatments decreased the plasma 6-keto $\text{PGF}_{1\alpha}$ in a similar percentage. It seems that we might also use this model for prediction of the long-term cardiovascular toxicity (toxicity assessment will be conducted in the future).

Our work demonstrated that colonic mucosa PGE_2 was a reliable biomarker for efficacy prediction. By eliminating the ineffective compounds that failed to decrease the mucosa PGE_2 within 4 days, we expect to considerably increase the chance of success and reduce the R&D cost.

Fig. 6 Down regulation of plasma 6-keto $\text{PGF}_{1\alpha}$ with celecoxib treatment



Acknowledgements We sincerely appreciate Dr. Roderick H. Dashwood (Texas A&M University) for the manuscript reviewing, Dr. Vincent H. Tam (University of Houston) and Lawrence N. Kwong (University of Texas MD Anderson Cancer Center) for the professional advice. We thank our lab mates including Rashim Singh, Lijun Xie, Yifan Tu, Dinh Bui, Zuoxu Xie, and Lu Wang who helped us to collect the rat tissue. The work is supported by a CPRIT Grant (RP180863) and NIGMS Grant (GM-070737) to Hu.

Compliance with ethical standards

Conflict of interest The authors declare no potential conflicts of interest.

References

- Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. *Cell*. 1996;87:159–70.
- Galiatsatos P, Foulkes WD. Familial adenomatous polyposis. *Am J Gastroenterol*. 2006;101:385–98.
- Half E, Bercovich D, Rozen P. Familial adenomatous polyposis. *Orphanet J Rare Dis*. 2009;4:22.
- Knudsen AL, Bisgaard ML, Bulow S. Attenuated familial adenomatous polyposis (AFAP). A review of the literature. *Familial cancer* 2003; 2:43–55.
- Gupta RA, Dubois RN. Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2. *Nat Rev Cancer*. 2001;1:11–21.
- Zelenay S, van der Veen AG, Bottcher JP, Snelgrove KJ, Rogers N, Acton SE, et al. Cyclooxygenase-dependent tumor growth through evasion of immunity. *Cell*. 2015;162:1257–70.
- Greenhough A, Smartt HJ, Moore AE, Roberts HR, Williams AC, Paraskeva C, et al. The COX-2/PGE2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. *Carcinogenesis*. 2009;30:377–86.
- Sonoshita M, Takaku K, Sasaki N, Sugimoto Y, Ushikubi F, Narumiya S, et al. Acceleration of intestinal polyposis through prostaglandin receptor EP2 in Apc(Delta 716) knockout mice. *Nat Med*. 2001;7:1048–51.
- Kwong LN, Dove WF. APC and its modifiers in colon cancer. *Adv Exp Med Biol*. 2009;656:85–106.
- Corpet DE, Pierre F. How good are rodent models of carcinogenesis in predicting efficacy in humans? A systematic review and meta-analysis of colon chemoprevention in rats, mice and men. *Eur J Cancer*. 2005;41:1911–22.
- Zeineldin M, Neufeld KL. More than two decades of Apc modeling in rodents. *Biochem Biophys Acta*. 2013;1836:80–9.
- Moser AR, Pitot HC, Dove WF. A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. *Science*. 1990;247:322–4.
- van Boxtel R, Gould MN, Cuppen E, Smits BM. ENU mutagenesis to generate genetically modified rat models. *Methods Mol Biol*. 2010;597:151–67.
- Irving AA, Yoshimi K, Hart ML, Parker T, Clipson L, Ford MR, et al. The utility of Apc-mutant rats in modeling human colon cancer. *Disease Models Mech*. 2014;7:1215–25.
- Rajendran P, Johnson G, Li L, Chen YS, Dashwood M, Nguyen N, et al. Acetylation of CCAR2 establishes a BET/BRD9 acetyl switch in response to combined deacetylase and bromodomain inhibition. *Can Res*. 2019;79:918–27.
- Amos-Landgraf JM, Kwong LN, Kendziorski CM, Reichelderfer M, Torrealba J, Weichert J, et al. A target-selected Apc-mutant rat kindred enhances the modeling of familial human colon cancer. *Proc Natl Acad Sci USA*. 2007;104:4036–41.
- Femia AP, Soares PV, Luceri C, Lodovici M, Giannini A, Caderni G. Sulindac, 3,3'-diindolylmethane and curcumin reduce carcinogenesis in the Pirc rat, an Apc-driven model of colon carcinogenesis. *BMC Cancer*. 2015;15:611.
- Femia AP, Luceri C, Soares PV, Lodovici M, Caderni G. Multiple mucin depleted foci, high proliferation and low apoptotic response in the onset of colon carcinogenesis of the PIRC rat, mutated in Apc. *Int J Cancer*. 2015;136:E488–95.
- Ertem F, Dashwood WM, Rajendran P, Raju G, Rashid A, Dashwood R. Development of a murine colonoscopic polypectomy model (with videos). *Gastrointest Endosc*. 2016;83:1272–6.
- Yun C, Dashwood W-M, Kwong LN, Gao S, Yin T, Ling Q, et al. Accurate quantification of PGE2 in the polyposis in rat colon (Pirc) model by surrogate analyte-based UPLC–MS/MS. *J Pharm Biomed Anal*. 2018;148:42–50.
- Yun C, Yin T, Shatzer K, Burrin DG, Cui L, Tu Y, et al. Determination of 7alpha-OH cholesterol by LC-MS/MS: application in assessing the activity of CYP7A1 in cholestatic minipigs. *J Chromatogr B Anal Technol Biomed Life Sci*. 2016;1025:76–82.
- Mukherjee D, Nissen SE, Topol EJ. Risk of cardiovascular events associated with selective COX-2 inhibitors. *JAMA*. 2001;286:954–9.
- Antman EM, DeMets D, Loscalzo J. Cyclooxygenase inhibition and cardiovascular risk. *Circulation*. 2005;112:759–70.
- McAdam BF, Catella-Lawson F, Mardini IA, Kapoor S, Lawson JA, FitzGerald GA. Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: the human pharmacology of a selective inhibitor of COX-2. *Proc Natl Acad Sci USA*. 1999;96:272–7.
- Cannon CP, Cannon PJ. Physiology. COX-2 inhibitors and cardiovascular risk. *Science*. 2012;336:1386–7.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.