The Importance of Drug Concentration at the Site of Action: Celecoxib and Colon Polyp Prevention as a Case Study

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ABSTRACT

Celecoxib is among the more potent and better clinically studied, nonsteroidal anti-inflammatory drugs (NSAID) for use as a chemoprevention agent for colorectal cancer. Its use is associated with a 40% to 50% response rate for reduction in adenomatous polyps. However, rare serious cardiovascular effects and even death with celecoxib and other NSAIDs make it important to understand why some patients respond and others do not. Celecoxib is a selective inhibitor of COX-2. Its anticancer mechanism has largely been attributed to the inhibition of COX-2. Celecoxib also shows activity to induce apoptosis in cancer cells not expressing COX-2. This includes activity to upregulate 15-lipoxygenase-1 (15-LOX-1) independent of COX-2 and increase the synthesis of 13-S-hydroxyoctadecadienoic acid (13-S-HODE) from linoleic acid (LA) to downregulate PPAR-δ and induce apoptosis in colorectal cancer models. In examining the effect of celecoxib on 15-LOX-1 for reducing adenomatous polyps in patients with familial adenomatous polyposis (FAP), Yang and colleagues point out the potential importance of drug bioavailability in blood, normal, and neoplastic colorectal tissue in patient response.

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Regular use of nonsteroidal anti-inflammatory drugs (NSAIDs) is associated with a 20% to 40% reduced risk of colorectal neoplasia (1). NSAIDs inhibit COX-2, the inducible prostaglandin endoperoxide synthase that converts the ω-6 polyunsaturated fatty acid (PUFA), arachidonic acid (ARA), to proinflammatory prostaglandins and found overexpressed in colorectal neoplasia. However, despite prevention benefit of upwards of 50% with the COX-2 selective inhibitors including celecoxib (2) and significantly less gastrointestinal toxicity compared with nonselective NSAIDs (3), the risk of rare, but serious cardiovascular events (4) constrains use for cancer prevention.

In an effort to identify factors that predict response to celecoxib for preventive use in patients with familial adenomatous polyposis (FAP), including whether upregulation of 15-LOX-1 is predictive, Yang and colleagues (5), discovered that celecoxib levels were significantly lower in polyp tissue than paired normal colorectal tissue or serum. They also observed that drug levels were higher in tissue in those patients who showed a response to treatment. To our knowledge, this is the first study to assess celecoxib levels in colorectal tissue and polyps of patients with FAP after treatment. Interestingly, these authors also assessed celecoxib levels in the $APC^{\Delta 580}$ mouse model of FAP and found drug levels to similarly be lower in tumor than normal tissue. Combined, these results indicate differences in the bioavailability of celecoxib between normal and neoplastic colon tissue and suggests that differential availability in neoplastic tissue may determine individual response.

Celecoxib is absorbed by the gastrointestinal tract and taken up into circulation undergoing methyl-hydroxylation to hydroxyl celecoxib in the liver principally by cytochrome P450 (CYP) 2C9 followed by oxidation to inactive metabolites (**Fig. 1**; ref. 6). In earlier work, effects of CYP2C9 genotype were investigated as modifiers of celecoxib efficacy for colorectal neoplasia, but the effects were found to be small (7). Findings of similar blood celecoxib levels between responders and nonresponders in the Yang study (5) indicate that circulating drug levels also do not predict individual response. In a recent study of volunteers undergoing repeat colonoscopy and tissue sampling after a single oral dose of 200 mg celecoxib, normal colon tissue levels of celecoxib were found to be high and to increase even after blood levels dropped (8). Together, these data support the disposition of celecoxib to colonic tissue with oral dosing and provide evidence for the potential of uncoupling from blood levels. Interestingly, in Yang and colleagues (5), celecoxib levels in blood and normal tissue correlated in responders only. Unclear are the reasons for lower polyp drug levels and absence of correlation between normal tissue, blood, and polyp drug levels in nonresponders.

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Cancer Prev Res 2022;15:205–8

doi: 10.1158/1940-6207.CAPR-21-0524

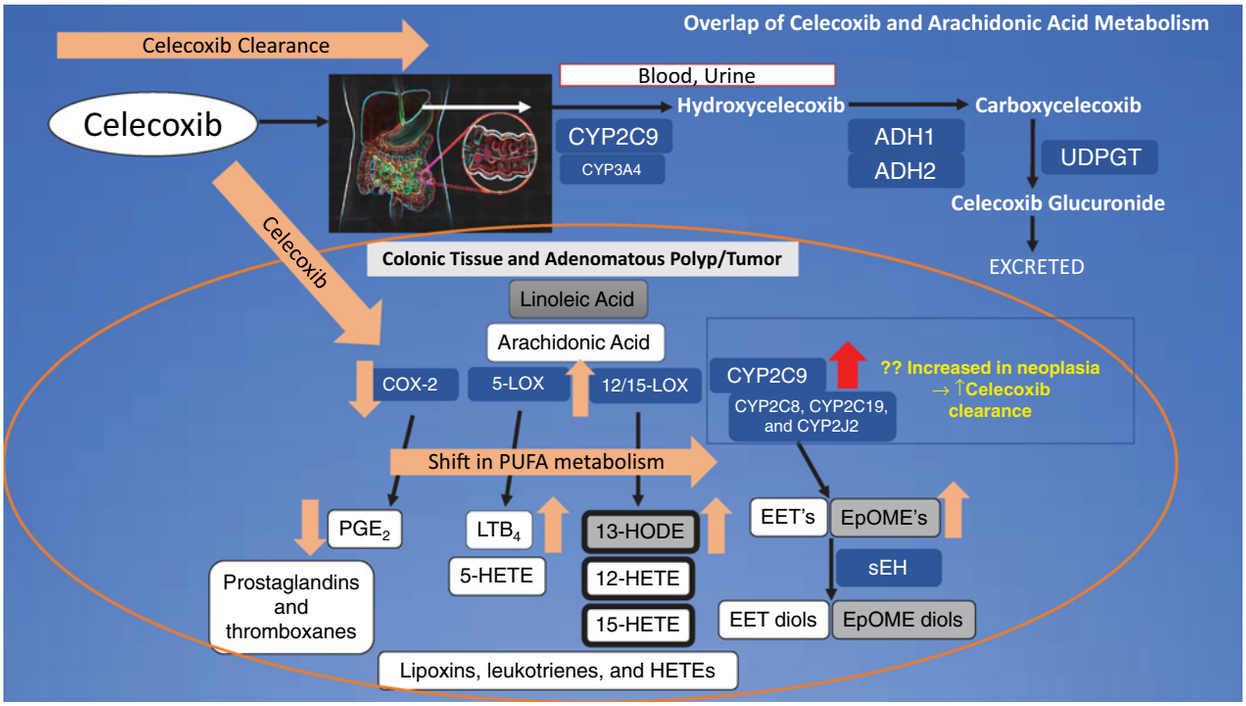

Figure 1.

Celecoxib metabolism and activity. Celecoxib is taken orally, is quickly absorbed, and reaches peak serum concentration in 1 to 3 hours, less than 3% is eliminated via feces and urine unchanged (18). Celecoxib then undergoes extensive metabolism in the liver via CYP2C9, with CYP3A4 playing a minor role (<25%), to hydroxycelecoxib. Hydroxycelecoxib is further oxidized by cytosolic alcohol dehydrogenases ADH1 and ADH2 to form carboxycelecoxib, which is then conjugated with glucuronic acid by UDP glucuronosyltransferases (UDPGT) for excretion. None of the metabolites are pharmacologically active. Celecoxib's target is inhibition of COX-2 (both in the liver and at the tissue level) which in turn reduces the production of proinflammatory prostaglandin E2 (PGE2). Less appreciated is a shift in PUFA metabolism after celecoxib exposure, with increases in LA and ARA availability for LOX and CYP450 metabolism as evidenced by increases in their products [e.g., 13-HODE (5), and EETs and EpOMEs (17)]. Unknown is whether this shift also alters celecoxib bioavailability via the increase in CYP2C9. LA products are colored grey, arachidonic acid products are colored white. Enzymes are blue. Thick black line indicates oxylipins quantified by Yang and colleagues (5). Only the specific oxylipins discussed are illustrated here, otherwise major classes of oxylipins are noted.HETE, hydroxyeicosatetraenoic acid.

Upregulation of several CYPs has been reported in tumors (9). CYP2C9 is reported to be upregulated in chemically damaged, metaplastic, and neoplastic tissues with evidence for increasing expression with tumor progression. Enayetallah and colleagues (10) reported that CYP2C9 protein levels were detectable in approximately 75% of human colon cancers (13 of 17) but not in four available matched normal tissues. This small study suggests that CYP2C9 may be upregulated in some colon cancers and related to stage of disease. However, in a study of colorectal tissues from the ColoCare Study, analyses of gene transcripts showed wide variability in CYP2C9 expression with no difference between normal and tumor (11). Similarly, analyses of CYP gene expression in The Cancer Genome Atlas showed large variability in normal and in colon tumor tissue and no significant difference (12). These results are consistent with the evidence that the expression and the activity CYP enzymes are regulated by numerous factors (13) explaining why the 'expression patterns' of CYPs, including CYP2C9 in extrahepatic tissues like the colon, remain poorly characterized.

Considering the results, we were also interested in the finding relating change in blood and tissue levels of the LOX-derived eicosanoid 13-S-hydroxyoctadecadienoic acid (13-S-HODE) following celecoxib treatment. Earlier preclinical evidence from the group showed that celecoxib, via non–COX-2 targeting upregulated the expression of 15-LOX-1, which in turn increased the metabolism of linoleic acid (LA) to 13-S-HODE and promoted antitumorigenic activity by inhibiting PPAR-δ in colon tumor models (14). In the clinical study, 13-S-HODE levels were found to be lower in polyps before treatment. After treatment, 13-S-HODE increased significantly in both the polyp and normal tissue of responders. In nonresponders, 13-S-HODE was unchanged or decreased slightly. Not shown was whether change in 13-S-HODE in normal or polyp tissue differed by drug levels.

Here we wanted to draw attention to another recent study highlighting the potential role of CYP monooxygenase-derived epoxygenated fatty acids (EpFA) in CRN. Recognizing that PUFA metabolism is deregulated in colon tumors, Wang and colleagues (12) quantified 56 EpFA in colon tumors from mice treated with azoxymethane (AOM) and dextran sodium sulfate

(DSS). In the AOM/DSS model, the CYP-derived EpFAs were increased in plasma and colon tumor tissue in parallel with elevated expression of several CYPs in tumor tissues. High levels of CYP expression were also observed in human colon cancer cell lines. Genetic knockout studies of relevant CYPs were shown to attenuate inflammation in the model, and pharmacologic inhibition of CYP monooxygenases suppressed tumorigenesis. As further evidence, treatment of AOM/DSS mice with ω-6 LA–derived 12, 13-epoxyoctadecenoic acid (12(13)-EpOME), but not ω-6 ARA–derived epoxyeicosatrienoic acids (EET), exacerbated inflammation and increased tumor size and number. These data suggest that CYP monooxygenases involved in LA and ARA metabolism may be upregulated in colorectal neoplasia. Unknown is the relevance of CYP monooxygenase or the derived EpOMEs in FAP patients as modifying factors for celecoxib activity.

With some exceptions (i.e., ref. 15), how the fatty-acid substrates compete across the multi-enzyme PUFA metabolic pathway to influence enzyme expression, their products, and their biological effects are not well understood. Indeed, we have wondered if the differential expression of the PUFA metabolic enzymes, in combination with diet, influence individual response to NSAIDs. Groups using targeted metabolomics have now identified "NSAID induced" alterations in lipid profiles in circulation (16). In our own study of patients who received celecoxib or placebo to prevent colorectal adenoma, we assessed celecoxib effects on circulating lipid profiles for effects on blood pressure. We postulated that some of the adverse effects of celecoxib may be related to sustained perturbations of PUFA metabolism following prolonged COX-2 inhibition. On prior studies, this would be expected to increase the availability of ARA and LA. In our study, while we did not observe any differences in 13-S HODE levels in circulation after 12 months of celecoxib compared with placebo, we did observe higher circulating levels of CYP monooxygenase-derived EpOMEs from LA and EETs from ARA (17). This included 12 (13)-EpOME identified by Wang and colleagues (12), as proinflammatory in the colitis model. We do not know if similar shifts are observed at the tissue level as fresh tissue collection was not practical in our trial. Like others, our results suggest that NSAID use globally alters PUFA metabolism (**Fig. 1**) for which any effects (especially predictable effects) on CYP monooxygenase activity and drug clearance generally, and at the tissue levels, are currently unknown.

We wanted to spotlight the current study to recognize the contribution of Yang and colleagues (5) in demonstrating the importance (yet challenging) of direct measures of drug and drug effects in clinical specimens in evaluating drug benefit for specific indications. Furthermore, we wanted to highlight advances in metabolomic profiling and their uses in characterizing global PUFA metabolism as tools to better characterize drugs and diet effect on PUFA metabolic enzymes. We think these tools will be especially important in the context of clarifying the effects of celecoxib and other NSAIDs for effects on these pathways and as cancer prevention agents.

Authors' Disclosures

No disclosures were reported.

Received November 23, 2021; revised January 24, 2022; accepted February 28, 2022; published first April 4, 2022.

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