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Novel Therapeutics: NSAIDs, Derivatives, and Phosphodiesterases

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Abstract

The chemopreventive efficacy of nonsteroidal anti-inflammatory drugs (NSAIDs) for colorectal cancer has been well documented. However, long-term use of NSAIDs is precluded owing to potentially fatal toxicities associated with their mechanism of action involving cyclooxygenase (COX) inhibition. But studies have shown that their anticancer activity may be due, in part, to an off-target effect. Cyclic guanosine monophosphate (cGMP) phosphodiesterases (PDEs), which are responsible for negative regulation of cGMP signaling, are an attractive COX-independent target. cGMP signaling is aberrantly suppressed in cancer cells and its activation appears to be sufficient to inhibit tumor cell growth. Chemically modifying sulindac has produced a series of new derivatives that lack COX-inhibitory activity but have improved cGMP PDE inhibitory activity. This approach is proving to be a promising strategy for the discovery of improved agents for the prevention and/or treatment of colorectal cancer.

Keywords

Sulindac; Colon cancer; Nonsteroidal anti-inflammatory drug; Cyclic guanosine monophosphate; Phosphodiesterase

Introduction

The anticancer activity of nonsteroidal anti-inflammatory drugs (NSAIDs) has been well documented [1–4]. In terms of colorectal cancer, NSAIDs have been shown to decrease mortality rate, slow disease progression, and inhibit de novo disease development [1–16]. A recent 10-year prospective study found that daily use of aspirin or non-aspirin NSAIDs reduced the risk of colorectal cancer in the normal population by as much as 35 % [17]. This protective effect was much more pronounced in high-risk patients, with daily NSAID use reducing colorectal cancer risk by as much as 60 % in patients who had a first-degree relative with colorectal cancer [17].

The biochemical mechanism through which the NSAIDs exert their anticancer activity is not fully understood, but it is clear that these drugs slow proliferation of and restore apoptotic

ability to colon tumor cells both in vitro and in vivo [3, 7, 18–22, 23••, 24]. Because inflammation is well known to promote tumor development and progression, many investigators attribute the anticancer activity of NSAIDs to their inhibition of the cyclooxygenase (COX) enzymes [1, 7, 14, 24–26]. However, several lines of evidence suggest that this may not be the case. For example, not all NSAIDs possess anticancer activity, and the rank order of potency for inhibition of tumor cell growth does not follow the rank order of potency for COX inhibition [23••]. Also, restoring COX activity does not rescue the cells from the anticancer activity of NSAIDs, and NSAIDs can inhibit growth of tumor cells that completely lack COX expression [18–20]. Most convincing is the ability of certain NSAID derivatives and metabolites to inhibit tumor development and progression despite lacking the COX-inhibitory activity of the parent compound [22, 25, 27, 28, 29•, 30•, 31].

A number of COX-independent targets have been identified as being involved in the anticancer activity of NSAIDs. Cyclic guanosine monophosphate (cGMP) phosphodiesterases (PDEs) are one such target. For example, we and others have shown that the potencies of certain NSAIDs for inhibition of colon tumor cell growth correlates with their potencies for inhibition cGMP PDEs in vitro [23••, 32], and certain NSAID metabolites and derivatives that lack COX-inhibitory activity but maintain anticancer activity also inhibit cGMP PDEs [23••, 32, 33].

In this review, we examine the emerging role of cGMP PDE isozymes and cGMP signaling in colorectal cancer development and progression. We also discuss recent and ongoing efforts to develop more effective and less toxic chemopreventive agents through a strategy that involves chemically modifying NSAIDs to enhance cGMP PDE inhibitory activity while eliminating COX-inhibitory activity.

cGMP Signaling

cGMP is an important second messenger within cells. As shown in Fig. 1, cGMP is formed through the activity of guanylyl cyclase (GC) from the precursor guanosine triphosphate in response to an extracellular stimulus such as a small peptide hormone such as guanylin or atrial natriuretic peptide, heat-stable enterotoxin, certain cytokines, or nitric oxide [34, 35]. Once formed, cGMP mediates its intracellular effects by modulating the activity of downstream effector proteins, which include cGMP-dependent protein kinase G (PKG), the cyclic nucleotide gated (CNG) ion channels, and certain PDE enzymes such as PDE2 and PDE3 [34, 36]. cGMP signaling is negatively regulated by cGMP-degrading PDE isozymes that catalyze the cleavage of the 3′–5′ phosphodiester bond of cGMP to produce guanosine 5′-monophosphate, thus determining the duration and overall amplitude of cGMP signaling in cells [34, 37–40].

The intracellular response that is elicited by cGMP signaling differs depending on the cell type [34]. This is largely due to differential expression of cGMP mediators between various cells and tissues. A prime example of this is salt handling by uroguanylin. In both the intestine and the kidney, uroguanylin, produced in response to high salt levels, directly activates type C GC (GC-C) to increase intracellular cGMP levels. Once formed, cGMP induces chloride secretion in the intestine by activating PKG II, which in turn opens the cystic fibrosis transmembrane regulator, but causes a decrease in sodium reabsorption in the kidney by closing CNG ion channels [41, 42]. Although these two activities are complementary, the mechanisms are fundamentally different owing to differential expression of cGMP signaling mediators. The exact effects of uroguanylin in the intestine are not possible in the kidney because renal cells lack expression of PKG II and cystic fibrosis transmembrane regulator. Conversely, the effects of uroguanylin in the kidney are

not possible in the intestine because intestinal epithelial cells lack the expression of the CNG channels that are expressed in the kidney. Furthermore, other cells of the body such as mammary epithelial cells do not respond to uroguanylin because they lack expression of the uroguanylin receptor GC-C [42].

cGMP PDEs

Cyclic nucleotide PDEs are a large superfamily of enzymes responsible for regulating second-messenger signaling by hydrolyzing the 3′–5′ phosphodiester bond in cGMP and/or cyclic adenosine monophosphate (cAMP). There are 11 PDE isozyme family members having different substrate specificity, regulatory properties, tissue localization, and inhibitor sensitivity [38]. PDE1, PDE2, PDE3, PDE10, and PDE11 are dual-substrate-degrading isozymes, whereas PDE5, PDE6, and PDE9 are cGMP-selective, and PDE4, PDE7, and PDE8 are cAMP-selective. In addition, each isozyme family contains multiple isoforms or splice variants in which over 100 have been suggested to exist in the human proteome. Depending on the PDE isozyme content of the target cell population and inhibitor selectivity, PDE inhibitors can increase the magnitude and/or the duration of cAMP and/or cGMP signaling. Increasing cyclic nucleotide levels can induce specific signaling pathways, which in the case of cGMP can activate PKG or other mediators (e.g., ion channels) to regulate cellular function and activities [36].

cGMP Signaling in Colorectal Cancer

Several studies have noted alterations within the cGMP signaling pathway in colorectal cancer [23•, 43–47]. One of the most well-characterized alterations involves decreased expression of the peptide hormones guanylin and uroguanylin in colorectal adenomas and adenocarcinomas that is accompanied by an increased expression of the guanylin receptor GCC [43, 48–50]. Other studies have found decreased expression of cGMP signaling mediators such as PKG and increased expression of negative regulators of cGMP signaling such as PDE in colon cancer [23•, 44, 51]. Whether the observed alteration is in cGMP formation, degradation, or downstream signaling, the outcome is largely the same—colorectal cancer cells generally have decreased basal levels of cGMP signaling and are hypersensitive to cGMP signaling activation when compared with the normal colonic mucosa, suggesting that suppression of cGMP signaling (e.g., from increased cGMP PDE expression) promotes tumorigenesis [23•, 47–49, 51].

Consistent with these observations, activation of cGMP signaling through direct activation of GC or PKG or inhibition of PDEs has been shown to have strong anticancer activity in various models of colorectal cancer [23•, 32, 33, 44, 46, 51–56]. For example, Waldman and colleagues [53, 57] have shown that activation of GC-C with endogenous or synthetic agonists significantly slows tumorigenesis both in vitro and in vivo by suppressing tumor cell proliferation. Activation of PKG has been shown to have an even more profound effect on colon cancer by inhibiting tumor cell proliferation, inducing tumor cell apoptosis, preventing tumor cell migration, and suppressing angiogenesis [23•, 51, 52]. Additionally, inhibition of PDEs, particularly PDE5, is sufficient to selectively induce apoptosis of colon tumor cells while having only minimal effects on normal colon cells [23•, 56]. Although the exact mechanism through which cGMP signaling activation inhibits colon tumorigenesis is not fully understood, it appears to involve inhibition of oncogenic β -catenin signaling [23•, 33, 58]. Whatever the mechanism involved, these studies suggest that targeting cGMP signaling may be an effective new strategy for the prevention and/or treatment of colorectal cancer.

NSAIDs Inhibit cGMP PDEs

As mentioned previously, NSAIDs have been shown to have strong anticancer activity, particularly for gastrointestinal cancers [1–16]. However, there is doubt as to whether COX inhibition, the primary mechanism responsible for both the anti-inflammatory activity and the potentially fatal toxicities associated with these drugs, is entirely necessary for their chemopreventive effects [18–20, 22, 23•, 24–28, 29•, 30•, 31]. Identifying and characterizing a COX-independent target could be an effective strategy for developing safer and potentially more effective anticancer agents.

One of the COX-independent mechanisms that has been proposed for the anticancer activity of NSAIDs involves inhibition of cGMP PDEs, resulting in increased intracellular cGMP levels and activation of cGMP signaling [23•, 30•, 32, 33]. Although not all NSAIDs inhibit cGMP PDEs, those that do have been shown to inhibit colon tumor cell growth in vitro with comparable and high potencies [23•, 32]. Additionally, altering cGMP signaling prior to NSAID treatment has been shown to alter the sensitivity of tumor cells to the anticancer effects of the drugs, suggesting that their anticancer activity is directly related to their effects on cGMP signaling [59, 60]. Unfortunately, the concentrations necessary to achieve such results are not clinically relevant owing to toxicity, and these effects on cGMP signaling have never been demonstrated in vivo. Nonetheless, with mounting evidence supporting an anticancer role of cGMP signaling, further investigation into the effects of NSAIDs on this pathway is warranted.

The Development of Novel NSAID Derivatives

Although several NSAIDs have been shown to inhibit cGMP PDEs, sulindac is one of the most active [23•, 32]. Additionally, studies have shown that the sulfone metabolite of sulindac retains both the cGMP PDE inhibitory and anticancer properties of the parent compound, despite lacking the ability to inhibit COX [22, 23•, 28, 31]. These findings demonstrate that the COX and cGMP PDE inhibitory activities of sulindac can be uncoupled, suggesting that the compound can be chemically modified to enhance cGMP PDE inhibitory and anticancer activities while minimizing COX-inhibitory activity.

Because sulindac sulfone lacked COX-inhibitory activity, initial efforts to design more potent sulindac derivatives focused on modifying the carboxylic acid moiety in which certain benzylamide derivatives were found to be active [33]. However, derivatives with modifications to both the carboxylic acid and sulfonyl moieties proved to be the most potent [33]. This approach was successful in producing a series of derivatives with significantly improved potency for inhibition of colon tumor cell growth and cGMP PDE inhibition [33]. However, these derivatives displayed limited oral bioavailability owing to poor absorption or metabolic instability, which precluded further development for chemoprevention.

In more recent efforts, our laboratory has used computer-aided drug design to design novel sulindac derivatives using the crystal structure of PDE5. Early studies involved docking of sulindac into the COX active site, which revealed that the carboxylic acid moiety is primarily responsible for COX inhibition [29•]. On the basis of these findings, more recent efforts have focused chiefly on substituting the carboxylic acid moiety with a positively charged (dimethylethyl)amino or (dimethylethyl)benzylamine [61]. This approach has yielded a number of interesting lead compounds that lack COX-inhibitory activity but have enhanced cGMP-PDE-inhibitory and anticancer activities [29•, 61•]. Even more promising, these new derivatives have shown promising pharmacokinetic and pharmacodynamic properties as well as anticancer efficacy in vivo; we continue to generate new compounds and formulations in order to further improve specificity to inhibit key PDE isozymes involved in tumorigenesis as well as to improve drug-like properties.

Conclusions

Colorectal cancer remains the second leading cause of cancer-related mortality in the USA [62]. Developing safer and more effective drugs for both the prevention and the treatment of colorectal cancer has the promise of improving the morbidity and mortality associated with this devastating disease. NSAIDs are a promising class of chemopreventive drugs because of their marked anticancer activity [1–4]. Although these drugs are known to cause potentially fatal toxicities as a result of their inhibition of COX enzymes, recent studies that demonstrate the ability to uncouple COX-inhibitory and anticancer activities suggest a new rationale for drug design using NSAIDs as scaffolds [23••, 24, 26, 29•].

cGMP PDEs are a promising new target for colorectal cancer chemoprevention for a number of reasons. First, several studies have shown that the expression of this family of proteins differs between normal and tumorigenic colon cancer cells [23••, 33]. Additionally, activation of cGMP signaling through either activation of GC or inhibition of cGMP PDEs has been shown to be sufficient to inhibit tumor cell growth and induce apoptosis [23••, 53, 57]. Furthermore, it is estimated that there are dozens of distinct cGMP PDE isoforms expressed within the human body, each of which is isolated to a distinct subset of cells and/or tissues [37, 38]. This differential expression pattern suggests that selective targeting of a single PDE isoform would have minimal systemic effects because of the limited tissue expression of that PDE isoform. In other words, using a drug to selectively target the PDE isoform expressed in colon tumor cells would have a low potential for side effects because there are likely few other tissues that also rely on the same PDE isoform without a means of compensation. This is demonstrated by the use of PDE5 inhibitors such as sildenafil and vardenafil for erectile dysfunction, a treatment strategy that has relatively little toxicity [39, 63].

Although using NSAIDs as a scaffold for designing new cGMP PDE inhibitors with anticancer activity is one approach, designing specific PDE inhibitors using high-throughput and in silico screening in another. Our laboratory is also using known PDE inhibitors as scaffolds. Unfortunately, the commercially available PDE inhibitors are largely ineffective at inhibiting colon tumor cell growth, probably owing to the lack of selectivity for the specific isozymes involved in tumor cell growth. However, we have developed a number of tadalafil derivatives through an approach similar to the one described here for sulindac which demonstrate promising anticancer properties [60, 64–69]. No matter what the approach, emerging data clearly demonstrate that targeting cGMP PDEs is a novel strategy for the development of safer and more effective drugs for the prevention and/or treatment of colorectal cancer.

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Fig. 1.

Cyclic guanosine monophosphate (*cGMP*) signaling. *GC* guanylyl cyclase, *5'GMP* guanosine 5'-monophosphate, *GTP* guanosine triphosphate, *PDE* phosphodiesterase, *PKG* protein kinase G