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Review article: mechanisms of action of mesalazine in preventing colorectal carcinoma in inflammatory bowel disease

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SUMMARY

A series of epidemiological, experimental and preliminary clinical trials strongly suggest that mesalazine or 5-aminosalicyclic acid (5-ASA) may have antineoplastic and potentially prophylactic chemopreventive properties. It is assumed that mesalazine may have similar genetic and molecular targets as nonsteroidal anti-inflammatory drugs (NSAIDs), which is further supported by its close similarity with aspirin, differing only in its structure by the presence of an amino group at position 5 of the benzene ring. The putative chemopreventive actions include the inhibition of inflammatory cascades and/or reactions involved in cell growth and proliferation, such as cyclo-oxygenase (COX-1 and COX-2), which regulate cell proliferation through the formation of prostaglandins; lipoxygenase; nuclear factor κB (NFκB), responsible for the subsequent expression of pro-inflammatory molecules; MAP kinases and Bcl-2, as well as the activation of apoptotic processes, such as the stimulation of intestinal sphingomyelinase. The peroxisome-proliferator-activated receptor δ (PPARδ), which also regulates gene transcription, is thought to play a role in both inflammatory and non-inflammatory driven carcinogenesis. This may be another significant target. It is hypothesized that 5-ASAs may prevent the enhancing effect of prostaglandins on PPARδ binding to DNA by its COX inhibitory properties, decreasing proliferation of colorectal mucosal cells in non-inflammatory bowel disease patients with sporadic polyps of the large bowel.

INTRODUCTION

Colorectal cancer (CRC) is the second most frequent carcinoma in western countries.[1] Despite progress in the genetics and molecular biology of the disease, surgical techniques and advances in chemotherapy, the burden of morbidity and mortality still remains high. Primary and secondary prevention is regarded as an effective tool in fighting this disease. Sporadic colon carcinoma contributes up to 80% of all cases, and certain aspects of lifestyle, such as a high-fat, high-meat, low-vegetable diet, and decreased physical activity, have been identified as risk factors.[2] Additional defined risk groups include patients with hereditary nonpolyposis colorectal carcinoma (HPNCC), familial polyposis coli syndrome and chronic inflammatory bowel disease (IBD), whereby the disease manifests either genetically or due to chronic inflammation, in which chemoprevention may be useful.[3–5] In an epidemiological cohort of > 1500 patients of whom 70% were treated with 5-aminosalicyclic acid (5-ASA) (continuously or intermittently), colonic cancer was not found to be increased compared with the healthy background population.[6, 7]

The topic of this article is the mechanisms of action of pharmacological treatments with special reference to mesalazine (5-ASA) in preventing CRC in patients with IBD.

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EPIDEMIOLOGICAL STUDIES

Epidemiological evidence, although predominantly retrospective, demonstrates a reduced relative risk (RR) of

developing colon cancer when patients with long-standing IBD take sulfasalazine (SASP) or 5-ASA regularly.[8–10] In a review of the literature, a 30–50% lower risk of colon cancer has been shown in IBD patients taking SASP or 5-ASA, close to figures found with aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs).[11] In contrast to these studies, the 5-ASA/SASP trials were retrospective and rather limited with regard to patient numbers and observation time.

MECHANISMS OF THE ANTI-PROLIFERATIVE ACTION OF MESALAZINE: CYCLO-OXYGENASE (COX)-DEPENDENT AND -INDEPENDENT STEPS

Despite such drawbacks, a variety of data from experimental work, animal studies and preliminary clinical trials strongly suggest that mesalazine may have antineoplastic and potentially prophylactic (chemopreventive) properties, which are comparable with those found with aspirin and other NSAIDs.[12] Mesalazine shares similar molecular targets, interfering with inflammation, proliferation and/or apoptosis, as aspirin and other NSAIDs. This can be explained by the close molecular similarity of mesalazine and aspirin, in which the former differs only in structure by the presence of an amino group at position 5 of the benzene ring.

The mechanisms leading to hyperplasia, adenoma/polyps and/or dysplasia and finally cancer, involve early and late genetic and molecular events, such as mutations in the *APC*, *K-ras* and *p53* genes and the loss of 18q, the short arm of chomosome 18. Some steps may be common in the pathogenesis of both sporadic cancer and malignancies arising from IBD, whereas others may differ. Mutations in the *APC* gene normally are not present in the early steps of cancers arising from IBD, but are found to play a role in early phases in familial APC syndrome and sporadic cancer. Overexpression of cyclo-oxygenase (COX)-2 and mutations of *K-ras* and *p53* are found in both types of carcinoma formation.[5]

Other pathways leading to sporadic colon cancer include mutations in the mismatch repair (MMR) system[13] and, as very recently suggested, the epigenetic gene silencing of MMR genes by methylation.[14]

This article will focus on the actions of mesalazine on COX-dependent and COX-independent mechanisms and reactive oxygen species: the role of mesalazine in the MMR system has not yet been studied.

Figure 1 demonstrates that inflammatory and non-inflammatory steps, e.g. COX-dependent and COX-independent mechanisms are involved in regulating apoptosis and proliferation. These pathways do not appear to act independently, but may work synergistically as recently demonstrated in animal models of tumourigenesis. Colonic tissue from animals with chemically induced colon cancer (non-inflammatory) show higher numbers and larger tumours in the presence of additional inflammation induced by trinitrobenzensulphonic acid (TNBS) or acetic acid compared with controls with no inflammation.[15, 16]

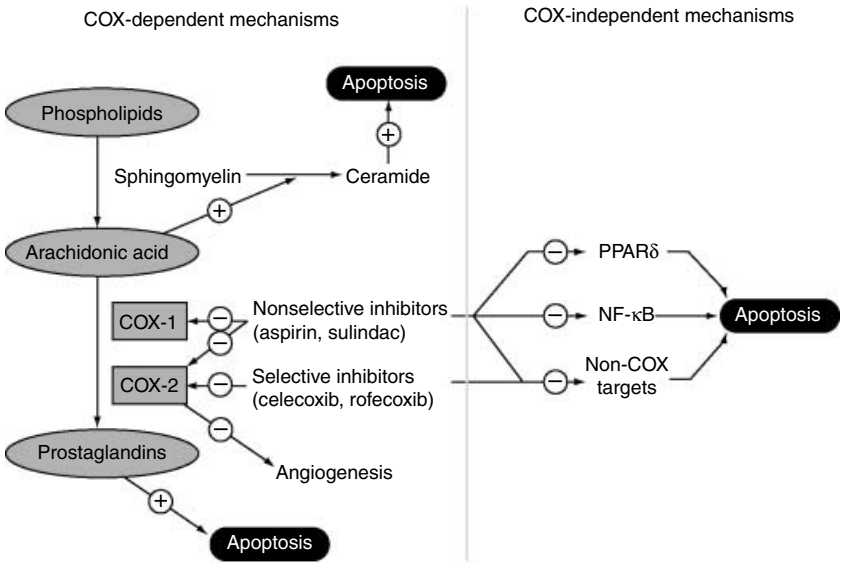

Figure 1. Schematic representation of COX-dependent and COX-independent mechanisms (adapted from Jänne and Mayer 2000 with permission from *New Eng J Med*[5]). COX, cyclo-oxygenase; PPAR, peroxisome proliferator receptor.

Recent experimental and preliminary clinical work has demonstrated that mesalazine may have *in vitro* and *in vivo* inhibitory properties comparable to other NSAIDs.[11, 17–19] Reversible inhibition of COX-1 and COX-2, NFκB activation, MAP kinases and Bcl-2 by mesalazine, was found in experiments using different cell systems including lymphocytes, polymorphonuclear leucocytes (PMNLs) and cultures from normal and neoplastic cell lines of animal and human origin.[20] In contrast to aspirin, which was shown to inhibit COX irreversibly, mesalazine (and other NSAIDs) inhibit COX and other steps (e.g. Bcl-2) reversibly. The molecular details for the majority of these reactions are only partly known, but recent work has shed light on some of these. Thus, inhibition of NFκB activation is most likely to be mediated by inhibition of IκB degradation, the inhibitory unit of the NFκB complex. It is worth noting that mesalazine has rather unspecific COX inhibitory properties with no preference for COX-2.

The concentration of mesalazine required to inhibit COX by 50% (IC_{50}) is mostly in the micromolar range (50–1000 μM), the IC_{50} for the inhibition of MAP kinases (ERK1/ERK2), c-jun-N-terminal kinases (JNK/SAPK), the inhibition of the TNF-α stimulated translocation of NFκB/Rel to the nucleus, and the inhibition of TNF-α stimulated (and IL-1) degradation of IκB, were found to be in the millimolar range (2.0–20 mM). In a recent clinical study in patients with ulcerative colitis, treatment with mesalazine for 8 weeks resulted in a strong inhibition of NFκB activation in biopsy specimens.[21] It has also been shown that mesalazine inhibits TNFα-mediated effects on intestinal cell proliferation and activation of MAP kinase and NFκB.[22] Furthermore, sulfasalazine suppresses NFκB activation by inhibiting the IκB kinases (IKKs).[23]

The clinical relevance of these findings is supported by the fact that the IC_{50} of these actions are in the same (mM) concentration range or even one or two orders of magnitude below the free mesalazine concentrations found intraluminally and intramucosally in IBD patients receiving mesalazine maintenance treatment (7.0–14.0 mM).[24]

An attractive hypothesis for the mesalazine/NSAID mode of action in this context relates not to COX inhibition itself, but rather the accumulation of arachidonic acid, which may be the decisive antiproliferative mechanism: arachidonic acid activates alkaline sphingomyelinase, which leads to increased ceramide production with activation of caspases and other apoptotic reactions.

A hypothesis linking COX-dependent and COX-independent inhibitory mechanisms together is based on the peroxisome proliferator-activated receptor δ (PPARδ) system as a common pathway for both inflammatory (COX-dependent) and non-inflammatory (COX-independent) driven carcinogenesis. Whereas COX inhibition by mesalazine, with the resulting decrease in production of eicosanoids, is well established, the inhibition by mesalazine of ligand-activated PPARδ binding to DNA, in promoter genes involved in cell proliferation/apoptosis, remains to be shown.

MECHANISMS OF ANTIPROLIFERATIVE ACTION: INHIBITION OF REACTIVE OXYGEN METABOLITES AND OXIDATIVE DNA DAMAGE

Reactive oxygen metabolites (ROMs) are produced during acute, and to a lesser extent, chronic inflammation, such as IBD. Major production sites include the inflammatory, endothelial and epithelial cells, including the mucosa. The most important ROMs are derived from the superoxide species, the extremely reactive hydroxy radical and the hypochlorite anion. A series of studies have demonstrated that mesalazine is able to inhibit these ROM species.[25] Activated monocytes, PMNLs and other ROM-producing cells participate in enhancing tissue and cell damage by interfering with metabolic pathways and macromolecular structures, such as the activation of lipid peroxidation and the induction of oxidative DNA damage, which is increasingly believed to be involved in carcinogenesis. These sites of inhibition are highlighted in Figure 2.

A series of arguments support the assumption that oxidative DNA-damaged products, especially the hydroxylated purine and pyrimidine bases (8-OH guanine, 8-OH adenine, 5-OH uracil and other hydroxylated base products), may play an important role in the early stages of carcinogenesis.[11] Elevated levels of these compounds were found in tissue and peripheral blood cells from patients with cancer and precancerous lesions, such as breast, gastric and colonic tissue, including that from IBD patients. The fact that oxidative damage may impair DNA repair and replication fidelity[26] and the observation that increased oxidative products are present in the gut lumen of patients with IBD and cancer, corroborates an important involvement of hydroxylated DNA bases in carcinogenesis.

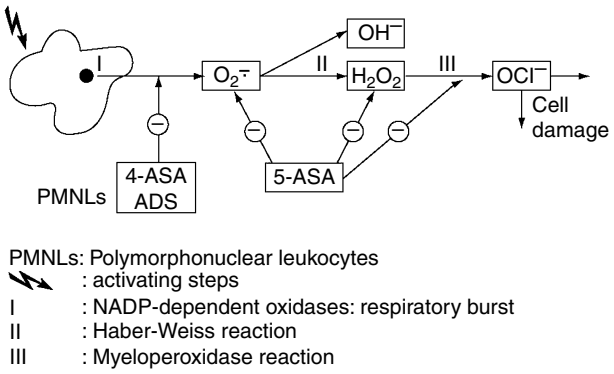

Figure 2. Sites of radical scavenging of 5-aminosalicylic acid (5-ASA) and its derivatives polymorphonuclear leucocytes (PMNLs).

Experiments from our laboratory, using electron spin resonance (ESR) spectroscopy with spin trap techniques to stabilize the short-lived superoxide and hydroxyl radicals, show that mesalazine is able to inhibit ROM production by scavenging superoxide and hydroxyl radicals (Figure 3), in a dose-dependent fashion.[25, 27]

One clinically important consequence of ROM scavenging by mesalazine is the subsequent attenuation or even complete inhibition of ROM-induced DNA damage. In a system containing Chelex-treated DNA from calf thymus, as a pharmacological model of human colonic DNA, and Fenton reagents (iron and hydrogen peroxide) to imitate inflammation, DNA damage increased significantly in the presence of ROM as shown by the increase of hydroxylated adenine, guanine and thymine bases. Mesalazine (5.0 mM) decreased the production of damaged products by 90%, which could be attributed to the almost complete inhibition of the Fenton reaction by mesalazine, which was monitored by ESR.[25, 28]

From these observations, it was important to demonstrate clear dose-dependent effects with regard to the

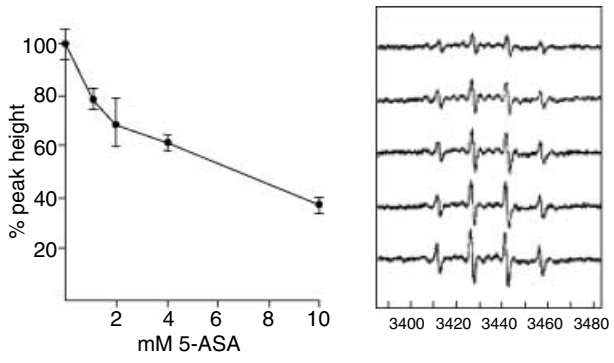

Figure 3. Effect of 5-aminosalicylic acid (5-ASA) on radical-generated electron spin resonance (ESR) signals.[25, 27]

IC_{50}. In a series of experiments using gas chromatography combined with mass spectroscopy and selected ion monitoring (GC/MS with SIM) we demonstrated in our model of human colonic mucosa that mesalazine dose-dependently inhibits ROM-induced DNA damage, as assessed by the diminished production of hydroxylated base products.[29]

The IC_{50} of these inhibitory reactions ranged from 0.05 to 2.0 mM for mesalazine, which correlates with concentrations found in the gut lumen of patients on mesalazine maintenance treatment. A clinical study performed in IBD patients treated with mesalazine for 1 month showed that the amount of hydroxylated DNA bases decreased significantly, supporting the concept that mesalazine may also have beneficial effects in clinical practice.[30] However, definitive conclusions from *in vitro* findings are not always completely correct, as effective concentrations maybe dependent on a variety of factors including, metabolic inactivation, i.e. the N-acetylation of free (active) mesalazine to N-acetyl-mesalazine (N-acetyl-aminosalicylic acid). Clinical and pharmacokinetic data support the view that the effective local (luminal) concentrations are sufficient for the above-described actions. It is also worth noting that inflammatory cells in contrast to intestinal cells do not contain N-acetyl-transferase and therefore may not be able to inactivate mesalazine.[27]

CONCLUSIONS

Clinical and experimental *in vitro* and *in vivo* data suggest that the aminosalicylates, including mesalazine, exhibit inhibitory actions in the growth of colorectal tumours. The precise mechanism of action is unknown but appears to occur through the inhibition of COX-dependent (inflammatory) and COX-independent (non-inflammatory) processes. This inhibitory action is also associated with the inhibition of steps involved in cell proliferation and/or apoptosis and the inhibition of oxidative DNA damage.

These *in vitro* and *in vivo* observations appear to have clinical relevance for treating CRC. The IC_{50} required to inhibit these COX-dependent and COX-independent regulators of inflammatory and malignant cell growth has been shown to correlate with the range of luminal concentrations normally achieved in patients on mesalazine maintenance treatment.

Despite epidemiological and pharmacological evidence of chemopreventive action, further studies are needed to

clarify the exact role of mesalazine in the chemoprevention of colonic cancer in IBD and in sporadic carcinoma (e.g. dose and magnitude of prevention). Prophylactic treatment with mesalazine may be possible for colon carcinoma and malignancies associated with IBD due to the favourable safety profile associated with the aminosalicylates.

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