

Systematic review: molecular chemoprevention of colorectal malignancy by mesalazine

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SUMMARY

Background

Mesalazine (mesalamine) (5-ASA) is considered an anti-inflammatory drug for the treatment of inflammatory bowel disease. It is well tolerated by most patients and induces mucosal healing specifically in ulcerative colitis. Besides its anti-inflammatory properties, 5-ASA has been studied for cancer inhibitory activities as it seems to reduce colorectal cancer incidence in patients using this drug for long periods of time. However, detailed molecular mechanisms of drug action are vague.

Aims

To evaluate known molecular mechanisms of 5-ASA on chemoprevention of colorectal malignancy.

Methods

Systematic review with search terms '5 aminosalicylic acid, mesalazine, 5-ASA, mesalazine, molecular mechanisms, chemoprevention' between 2006 and August 2009.

Results

A total of 48 studies were retrieved that link 5-ASA chemopreventive properties to five distinct pathways. These include interference with cell cycle progression (12 references), scavenging of reactive oxygen- or nitrogen-derived metabolites (16 references), TNF- α /TGF- β signalling (11 references), WNT/ β -catenin signalling (5 references) and anti-bacterial properties (4 references).

Conclusions

In the recent years, a large amount of molecular data has accumulated supporting the notion that 5-ASA biological effects interfere with colorectal cancer development. These molecular pathways are of special interest in the search for 5-ASA's molecular target(s) and development of novel chemopreventive compounds.

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INTRODUCTION

The term chemoprevention refers to the use of natural or synthetic substances to reverse, suppress or delay the risk of developing any disease. In most cases, however, this term is applied to the process of carcinogenesis and the current use of this name refers to a pharmacological approach to arrest and/or reverse cancer development and progression.^{1, 2} One medication that has received significant attention for its possible role as a chemopreventive agent is 5-aminosalicylic acid (5-ASA), which is a structural analogue of aspirin and a drug of choice for mild-to-moderate ulcerative colitis.³ 5-ASA has been derived from sulfasalazine which is one of the oldest anti-inflammatory agents used for the treatment of Crohn's disease and ulcerative colitis,^{4, 5} two major human forms of chronic inflammatory bowel diseases (IBD).⁶ 5-ASA reduces oxidative stress, inhibits cell proliferation and promotes apoptosis.^{7, 8} After oral or rectal administration, 5-ASA acts locally in the colon and is absorbed by colonic epithelial cells.⁹ The effectiveness of the drug is related to its mucosal concentration,¹⁰ the type of release formulation (e.g. pH-dependent) and the individual intraluminal pH as well as motility.¹¹ On a molecular level, 5-ASA inhibits cyclooxygenase-2 (COX-2)/prostaglandin E2 synthesis,^{12, 13} decreases transcriptional activity of NF- κ B by modulating RelA/p65 phosphorylation¹⁴ and interferes with Wnt pathway through protein phosphatase 2A (PP2A).¹⁵

Although clinical evidence for chemopreventive effects of 5-ASA in patients with IBD suggests specific anti-inflammatory or oxygen scavenging properties, this drug seems to possess multiple modes of action which, despite numerous experimental studies, remain partially unknown. Recent studies on the incidence of colitis-associated cancer in IBD have clearly demonstrated that the overall incidence of this complication has not decreased within the past decades.¹⁶ Thus, prevention of malignancy is still an important issue in such patients.

METHODS

As many novel results appeared in the literature after the review by Stolfi *et al.*⁸ (that was submitted by March 2008), the current article will emphasize the major progress made since then. A systematic literature search of the PubMed, Scientific Commons, Med-Resource Reviews Database, MedscapeCME, Ingenta,

Scirus, British Library Direct databases was conducted. Results were limited to full papers published in English language between January 2006 and September 2009 to identify chemopreventive properties of 5-ASA on molecular level. Studies were selected for inclusion when the following criteria for the search terms were met: 5 aminosalicylic acid, mesalamine, 5-ASA, mesalazine, molecular mechanisms and chemoprevention. Some relevant publications of early dates were also cited when background information required consideration. The initial search for 5-ASA and synonyms yielded 422 original papers and 127 review articles. After subtracting reviews and non-English papers, 384 publications remained. We then narrowed down the search by filtering only those studies with molecular mechanisms and chemoprevention. Finally, we excluded duplicates and obtained 48 relevant publications, which we reviewed in the current manuscript. These reports link 5-ASA's molecular activities to certain pathways (Figure 1).

5-aminosalicylic acid interferes with cell cycle progression

Cell cycle regulation represents one of the defence mechanisms that stabilize the integrity of the genome.¹⁷ Mediated through specialized checkpoint proteins, cell cycling provides additional frame of opportunities to correct, change or destroy cell aberrations on transcriptional and translational levels.¹⁸ Unexpected activation of cancer-prone mechanisms, however, may deregulate, disrupt or inhibit normal cell cycle resulting in accumulation of genomic errors and eventually leading to malignant transformation or cell death. Recent findings suggest that 5-ASA interferes with cell cycle on different levels. 5-ASA inhibits the proliferation of colon cancer cells resulting in S-phase arrest and upregulation of Chk1 and Rad17, but not ATM proteins.¹⁹ The activation of replication checkpoints by 5-ASA may therefore prolong DNA replication and improve DNA replication fidelity thus reducing mutation rate.²⁰ This effect was shown to be independent of p53; however, p53 revealed strong phosphorylation at Ser15 residue upon exposure to 5-ASA. Importantly, the position of the amino group in 5-ASA benzene ring is critical for improvement of replication fidelity.²¹ As p53 activates epidermal growth factor receptor (EGFR) expression in cancer cells²² and EGFR function is required for cell cycle progression^{23, 24} there are many reasons to suggest a

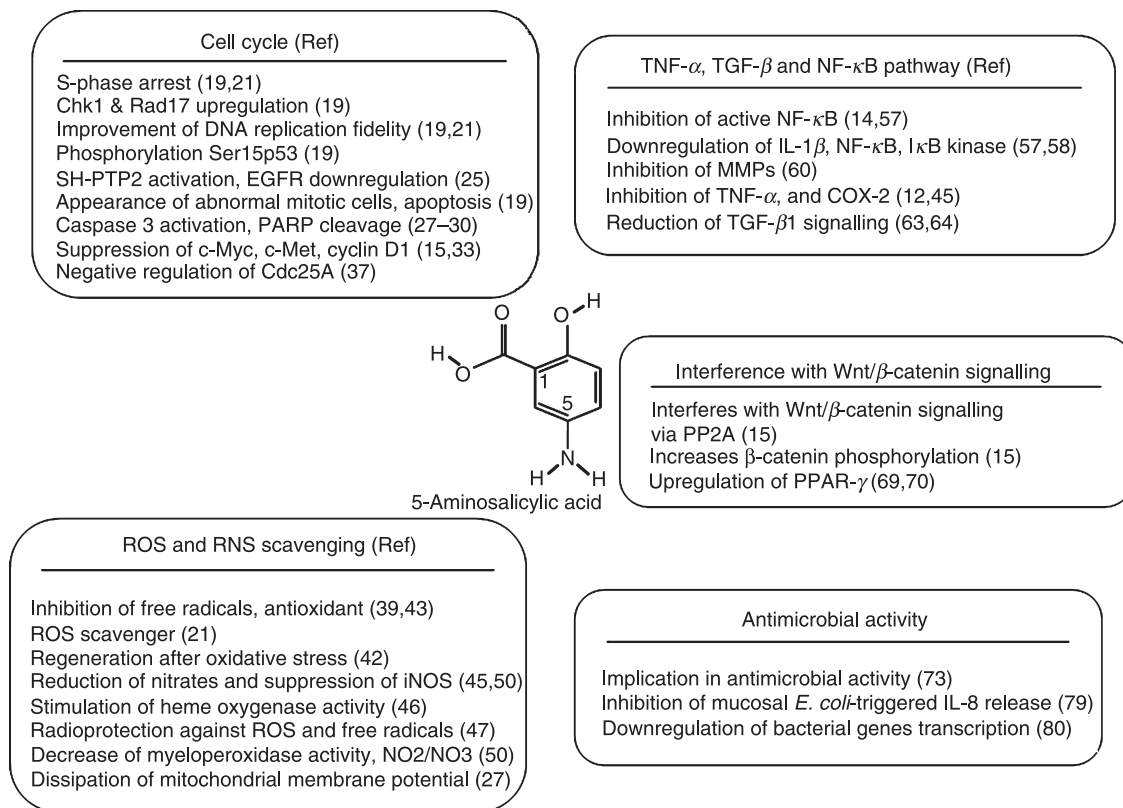


Figure 1. 5-aminosalicylic acid's molecular chemopreventive properties. The molecular actions that relate to 5-ASA's potential chemopreventive activity can be structured to five different pathways. Both cell cycle and Wnt-signalling seem specifically interesting to colorectal cancer chemoprevention.

connection between 5-ASA-mediated phosphorylation of p53 and EGFR status. Findings of Monteleoni's group show 5-ASA inhibition of EGFR signalling pathway by enhancing activity of SH-PTP2 phosphatase.²⁵ Not surprisingly, EGFR is now considered an important target for cancer therapy.²⁶ One may expect therefore that 5-ASA-mediated suppression of EGFR signalling also induces apoptosis. Indeed, recent studies revealed that 5-ASA was able to trigger both caspase-dependent and independent apoptotic pathways.²⁷ This seems to be dependent on the cell doubling time and, to a lesser extent, on the concentration of 5-ASA used for the study.²⁸ Therefore, slower growing cells should delay 5-ASA induced S-phase arrest. Studies of Koe-link *et al.*, however, suggest that the dosage of 5-ASA may affect the cellular response.²⁹ Our own experimental data confirm that at higher concentrations, 5-ASA increased apoptosis and induced the appearance of abnormal mitotic cells.¹⁹ Significant increase in caspase-3 activity, cleavage of PARP and caspase-8

was also observed in CaCo-2 cells³⁰ thus supporting the 5-ASA effects on inhibition of cell growth and induction of apoptosis.

Another line of evidence explaining 5-ASA chemopreventive properties involves c-Myc oncoprotein, which prevents cell cycle arrest in responses to growth-inhibitory signals, differentiation stimuli, or mitogen withdrawal.^{31, 32} 5-ASA downregulates c-Myc gene and protein expression resulting in the cell growth inhibition and apoptosis.³³ In light of recent publications showing c-Myc involvement in DNA replication and the S-phase checkpoint processes,³⁴ these data corroborate the above findings of improvement replication fidelity by 5-ASA²⁰ hence representing another mechanism of cancer chemoprevention.

In eukaryotes, activation of cyclin/cyclin-dependent kinases (CDKs) is an important function of the cell cycle machinery that modulates entrance of cell into specific stage of division. Not surprisingly, blocking CDKs by specific drugs is in front line of new cancer

therapeutic strategies.^{21, 35} 5-ASA-mediated inhibition of β -catenin expression was recently shown to down-regulate cyclin D1, c-met and c-Myc genes.¹⁵ Another protein, Cdc25A, is rate-limiting oncogene that determines stability of the genome by controlling S-phase checkpoint³⁶ was recently implicated as 5-ASA target in CRC cells.³⁷ 5-ASA negatively regulates Cdc25A protein expression resulting in delayed progression of CRC. This regulation is independent of CHK1 or CHK2, but is mediated by 26S proteasomal pathway. Interestingly, inhibition of Cdc25A activity by a K vitamin analogue can induce a strong and prolonged activation of EGFR-MAPK pathway, which leads to suppression of transcription factors CREB and c-Myc and results in decreased expression of Cdc25A and cyclin D1 levels.³⁸ Overall, the above data on involvement of 5-ASA into the cell cycle progression suggest possible mechanism for 5-ASA chemoprevention.

5-aminosalicylic acid scavenges reactive oxygen and nitrogen metabolites

5-aminosalicylic acid is a very efficient scavenger of reactive oxygen species (ROS) and its inhibitory effects against free radicals have been described in the literature.³⁹ As inflammation and inflammation-driven malignancies are accompanied by accumulation of ROS, treatment with 5-ASA may effectively prevent these events, especially when applied in IBD. Although inflammation is an important biological risk factor for cancer development,⁴⁰ only recently has severity of inflammation been linked to carcinogenesis.⁴¹ 5-ASA and its metabolite N-acetyl-5-ASA show significant $\cdot\text{O}_2^-$ scavenging effects.²¹ Oxidized 5-ASA can regenerate by endogenous compounds, e.g. ascorbic acid, cysteine, or glutathione (GSH), which may preserve the drug effects in tissues undergoing oxidative stress.⁴² The antioxidant effect of 5-ASA was also shown on copper-mediated LDL oxidation.⁴³ In addition, studies in rats demonstrate that 5-ASA affects induction of inflammation, promotes mucosal healing and reduces tissue inflammation in chemically induced colitis.⁴⁴ Combined with another antioxidant N-acetylcysteine (NAC), 5-ASA reduces nitrate generation and suppress nitric oxide synthase (iNOS) activity.⁴⁵

The effects of 5-ASA on the expression and activity of haem oxygenase-1 (HO-1) have been evaluated in the colitis provoked by instillation of trinitrobenzene sulphonic acid (TNBS).⁴⁶ HO-1 can modulate colonic inflammation by endogenous anti-oxidant and

anti-inflammatory moieties. Intraluminal administration of 5-ASA induced colonic HO-1 protein expression and stimulated HO-1 enzyme activity suggesting that 5-ASA may exert its colonic anti-oxidant and anti-inflammatory effects *in vivo* in part through the upregulation of HO-1 enzyme expression and activity.

5-aminosalicylic acid may induce dissipation of mitochondrial transmembrane potential and enhances ROS production.²⁷ The authors suggest a mechanism of 5-ASA interference with colon carcinogenesis that converges on mitochondria and involves suppression of 5-ASA-induced peroxides by GSH, accumulation of mitochondrial proteins into the cytoplasm and activation of caspases-3. Also, 5-ASA can protect from radiation-induced ROS production and tissue damage.⁴⁷ The authors tested the radiation-protective effect of 5-ASA in mouse bone marrow. 5-ASA pre-treatment decreases death caused by radiation-induced gastrointestinal and haematopoietic syndromes. 5-ASA prevents radiation-induced depletion of antioxidant enzymes and lipid peroxidation, probably by quenching the hydroxyl radicals. The proposed mechanism is thought to involve inhibition of chromosomal aberrations and increased expression of p53 and p21 proteins. In the same study, authors performed Fenton's assay and showed ROS scavenging effects of 5-ASA.

Nitric oxide has been implicated in pathogenic mechanism of many diseases.^{48, 49} It acts either as a constitutive isoform of NO synthase (NOS) or inducible isoform of NOS (iNOS), both are involved in inflammatory processes. Treatment with 5-ASA decreased myeloperoxidase activity, serum nitrite/nitrate levels and iNOS expression.⁵⁰

TNF- α , TGF- β and NF- κ B pathway

Tumour necrosis factor alpha and beta are multifunctional proinflammatory cytokines with effects on lipid metabolism, coagulation, insulin resistance and endothelial function.⁵¹ The TNF pathway has been considered as an anti-cancer strategy since its discovery several decades ago.⁵² It acts through two receptors, TNFR1 which is expressed by all human tissues and is the major signalling receptor for TNF- α , and TNFR2 which is predominantly expressed in immune cells.⁵³ Nuclear factor kappaB (NF- κ B) signalling plays a key role in TNF expression and response.⁵⁴ Members of the NF- κ B family normally interact with inhibitors called I κ Bs or the unprocessed forms of NF- κ B1/2 and therefore remain inactive.⁵⁵ A number of signalling

molecules (e.g. antigen receptors, TNFRs and IL-1 cytokines) induce differential activation of NF- κ B heterodimers (Figure 2). TNF is also an important target of anti-inflammatory therapy in IBD.⁵⁶

In human colon epithelial cells, 5-ASA inhibits phosphorylation of Ser536 NF- κ B subunit p65, the critical residue for transcriptional activity of NF- κ B.^{14, 57} 5-ASA may equally inhibit I κ B/NF- κ B signalling activated by other factors, including interleukins. 5-ASA decreases serum IL-1 β production, tissue NF- κ B expression, IL-1 β -induced I κ B kinase activation and subsequently interferes with I κ B α degradation and p65 nuclear accumulation.⁵⁸ NF- κ B-mediated expression of matrix metalloproteinases (MMPs) has a central role in tumorigenesis.⁵⁹ 5-ASA inhibits MMPs expression via NF- κ B mediated cell signals and invasiveness.⁶⁰

Tumour necrosis factor- α seems to contribute to inflammation in IBD patients by initiation of intracellular signalling cascades resulting in release of transcriptional factor NF- κ B and activation of COX-2 both of which have protumorigenic activity.⁶¹ 5-ASA blocks both COX-2-dependent and -independent cancer cell growth. It can inhibit TNBS-induced TNF α overexpression⁴⁶ and TNF- α - or IL1- β -induced COX-2/prostaglandin E2 (PGE2) synthesis.¹² 5-ASA inhibition of interleukin-1-stimulated NF-kappaB RelA/p65 phosphorylation was proved to be in parallel with the decreased transcriptional activity.¹⁴

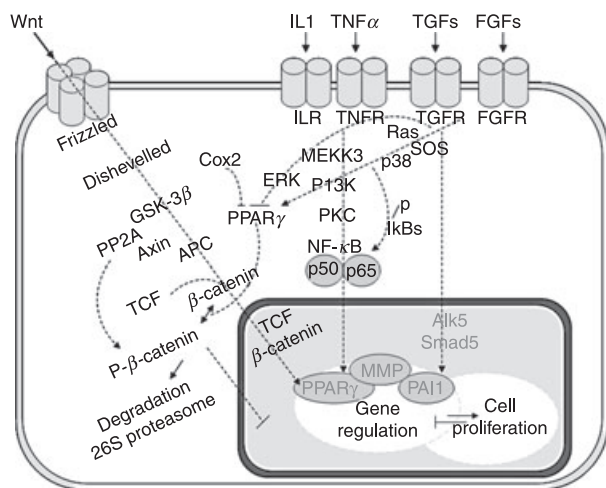


Figure 2. NF- κ B and Wnt/ β -catenin signalling pathways in colon cells. 5-ASA seems to interfere with all pathways that are displayed in this illustration leading to reduced downstream transcriptional activity.

Members of the transforming growth factor beta (TGF- β) family are cytokines that play multiple roles in autocrine and paracrine processes of tumorigenesis.⁶² Besides various TGF- β proteins, this family includes bone morphogenetic proteins (BMPs), activins and corresponding receptors. Recently, 5-ASA was shown to reduce the TGF- β 1 signalling response of CRC-derived cells, but not the BMP signalling.⁶³ 5-ASA also inhibits transcription of TGF- β -targeted gene plasminogen activator inhibitor-1 (PAI-1) which was proven to reduce polyp formation in mice.⁶⁴ 5-ASA reduction of TGF- β response acts through inhibition of aktivin-receptor-like-kinase-5 (ALK-5) and Smad nuclear localization.⁶⁴

Wnt/ β -catenin pathway

The Wnt signalling pathway is involved in embryonic development but may also contribute to tumour formation when aberrantly activated. That is why therapeutic interventions have been suggested to interfere with canonical Wnt/ β -catenin activity for prevention and/or treatment of CRC.⁶⁵ 5-ASA interferes with Wnt/ β -catenin pathway via protein phosphatase 2A (PP2A).¹⁵ In normally dividing cells lacking Wnt, β -catenin is phosphorylated via multiprotein destruction complex containing glycogen synthase kinase β (GSK3 β), axin, dishevelled, casein kinase 1, and adenomatous polyposis coli (APC) following its translocation and proteasome-dependent degradation in the nucleus. However, during tumorigenesis Wnt binds to Frizzled/low-density lipoprotein receptor complexes thus inactivating GSK3 β which results in β -catenin accumulation in the cytoplasm.⁶⁶ (Figure 2). 5-ASA activates PP2A which phosphorylates β -catenin thereby reducing its expression in the nucleus.¹⁵

The very recent study demonstrates 5-ASA involvement in the expression of an E-cadherin member mucin-1, which is able to sequester β -catenin on the plasmatic membrane of treated cells and hampers its function.⁶⁷

Tumour suppressor peroxisome proliferator activated receptor- γ (PPAR- γ) was shown to be regulated by the Wnt/ β -catenin pathway.⁶⁸ 5-ASA upregulates PPAR- γ expression and interferes with PPAR- γ active centre, which may explain some anti-neoplastic effects of this drug.^{69, 70} Interestingly, 5-ASA, sulfasalazine and rosiglitazone (a specific PPAR- γ agonist) significantly reduced the cellular expression of TC22, a novel human tropomyosin (hTM) isoform, which is expressed

in all tested colorectal carcinomas, but not in normal colon epithelial cells.⁷¹ This implies TC22 being modulated by PPAR- γ and suggests a novel anti-neoplastic molecular effect of mesalazine. New anti-inflammatory and anti-neoplastic compounds will probably use PPAR- γ as molecular target.

Another tumour suppressor, APC, is one of the major negative regulators of Wnt/ β -catenin pathway. Loss of APC function leads to accumulation of β -catenin and is thought to be an early event for cancer development, specifically in familial adenomatous polyposis (FAP). In the recent study of Koelink *et al.*, the novel FabplCre;Apc^{15lox/+} mouse model, which developed distally located tumours, to mimic human sporadic CRC development, was used to determine the effect of 5-ASA on the development of colorectal tumours.⁷² When administered as enemas, 5-ASA did not reduce spontaneous tumour development in this model of colitis-associated carcinogenesis.

5-aminosalicylic acid antimicrobial activity

5-aminosalicylic acid has also been implicated in antimicrobial activity.⁷³ There is increasing evidence that *E. coli* organisms are important in Crohn's disease pathogenesis.^{74–77} Some adherent and adhesive mucosal *E. coli* isolates are able to invade colonic polyps and cancers⁷⁸ resulting in activation of toll-like receptors, TNF, IL-8 and NF- κ B signalling. 5-ASA inhibits MAPK-dependent IL-8 release triggered by mucosal *E. coli* isolates in a dose-dependent manner.⁷⁹ Moreover, bacterial invasiveness was decreased by 5-ASA. Although the exact mechanism of 5-ASA antibacterial activity remains unknown, recent finding of 5-ASA downregulation of bacterial genes expression implicate intestinal bacteria as pharmacological targets of 5-ASA, perhaps contributing to the therapeutic action of

this important class of IBD drugs.⁸⁰ This effect could have arisen from the similarities among known 5-ASA targeted genes in eukaryotes and found in the homologous among commensal gram-negative enteric bacteria. These observations open new window to explain chemopreventive properties of 5-ASA in dysplastic epithelial cells progressing from dysplasia to cancer.

CONCLUSIONS

Although data on 5-ASA chemopreventive properties increase exponentially for the recent years, we are far from understanding the molecular pathways and mechanisms driving such events. It is still unclear how many metabolites can be evoked from 5-ASA and the precise pharmacokinetics of each of them. This review focused on the recent progress made in discovering novel mechanism of 5-ASA on molecular level that can potentially explain chemopreventive properties of this drug. Nevertheless, new strategies are needed to identify 5-ASA's molecular targets and, more importantly, unify the existing functions of this chemical. Combinatorial approaches embracing genomics and proteomics as well as system biology would benefit towards the above goal.

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