Journal of Crohn's and Colitis (2009) **3**, 149–156

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REVIEW ARTICLE

The long journey of salicylates in ulcerative colitis: The past and the future

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Received 21 February 2009; received in revised form 5 May 2009; accepted 5 May 2009

KEYWORDS
Ulcerative colitis;
Salicylates;
MMX mesalazine

Abstract

The advent of salicylates in the treatment of ulcerative colitis started in 1938 with the discovery of Salazopyrin by Nanna Svartz. This drug offered for the first time a therapeutic chance to patients with ulcerative colitis. In this paper we describe the fascinating history of Salazopyrin and salicylates from the first serendipitous observations to the last randomized clinical trials. Attention was paid to the pharmacokinetics and the mechanism of action of 5-aminosalicylates and, in particular, to the issue of the mucosal concentrations of 5-aminosalicylates and its therapeutic efficacy. Moreover a look at the new oral mesalazine formulations that allow the homogenous distribution of 5-aminosalicylate through all the large bowel was taken. Lastly, the possible use of mesalazine in the prevention of colorectal cancer was reviewed.

Contents

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doi:10.1016/j.crohns.2009.05.001

The treatment of ulcerative colitis (UC) until the early 20th century was empirical, anecdotical and inefficacious. It consisted of bed rest, low residue and high protein diet, vitamin supplement, blood transfusion, opioids and rectal instillation of various substances (hydrogen peroxide, silver nitrate, tannic acid, and bismuth). The prognosis of patients with UC at that time was poor and mortality was very high.

The discovery of Salazopyrin in 1938 significantly changed the fate of these patients offering them, for the first time, a therapeutic chance. In this paper we describe the fascinating history of Salazopyrin and salicylates from the serendipitous observations to randomized clinical trials.

1. Nanna Svartz and Salazopyrin

Salazopyrin (Sulphasalazine) was synthesized by Nanna Svartz (1890–1986), professor of Medicine at the Karolinska Hospital in Stockholm.

Moving from the hypothesis that both rheumatoid arthritis and ulcerative colitis (UC) were caused by streptococcal infection (diplostreptococci) and the inflammatory changes of UC began in the connective tissue of the submucosa, it was desirable to find a drug capable of concentrating in the connective tissue. She therefore decided to combine via an azo-bond Sulphapyridine (SP), a new drug very active on bacteria, and 5-aminosalicylic acid (5-ASA) active on connective tissue. With the help of a Pharmacia chemist, Philip Willsted, she was able to produce the compound Salicylazosulphapyridine named Salazopyrin (SASP)[1] (Fig. 1). Svartz noted that SASP was able to improve not only patients with rheumatoid arthritis, but also the associated ulcerative colitis in some patients. The recommended dose was two 500 mg tablets 4–6 times daily. At these high doses headache, nausea, vomiting, fever, cyanosis (methemoglobin), allergic reactions, jaundice, leucopenia and agranulocytosis were observed in 15–20% of patients.

Nanna Svartz continued to study SASP during the Second World War and published her first series of 124 patients in 1948.[2] This open study showed that most of the patients (70–80%) with mild–moderate UC responded well, but relapse was the rule at drug discontinuation. She suggested that SP was the active agent.

Bargen of the Mayo Clinic introduced SASP in the Unites States and published a large series of patients successfully treated with the new drug.[3] In Britain SASP became available only several years later after 1955 and at the beginning it was not well accepted by doctors and patients, in particular for its frequent side effects.

This was an era when the controlled therapeutic trials were not yet applied to the assessment of drug effectiveness.

2. Early controlled trials

The pioneer of the controlled clinical trials (RCTs) in IBD was Sidney Truelove, who in 1955 published the first RCT on the effectiveness of corticosteroids in severe UC.[4] Sidney

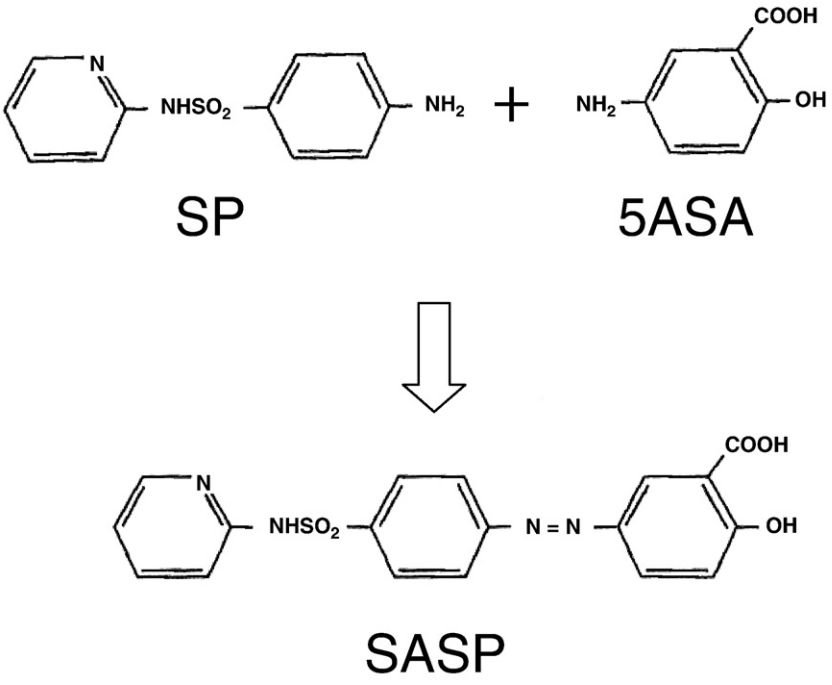

Figure 1 Chemical structures of Salazopyrin and its compounds, SP and 5-ASA, linked by an azo-bond.

Truelove encouraged a generation of British (and non) gastroenterologists to become involved in clinical trials by using a biometric approach based on randomization, control group and blind assessment of the results. He introduced the first disease activity index in UC (Truelove and Witts index) that is considered to be a milestone on the road of evidence-based medicine.[4]

The first double blind, RCT of Salazopyrin was conducted in 1962 at St Mark's Hospital, London, by Baron et al.[5] As it was postulated that the side effects of SASP were related to sulphapyridine it was considered the possibility of substituting sulphadimidine for SP, to reduce side effects. SASP 4 g/day and salicylsulphadimidine were therefore compared with placebo in induction of remission in mild cases of UC. SASP but not sulphadimidine resulted to be superior to placebo. In this study it was for the first time used as a biometric technique grading the severity of endoscopic mucosal changes. Side effects were significantly higher in the SASP group.

Subsequently the same group of St Mark's Hospital showed for the first time that SASP 2 g/day administered as maintenance therapy for one year was extremely more effective than placebo in preventing relapses rate.[6] A low incidence of side effects was observed due to the use of smaller dose. This was considered a very important clinical message as it was estimated that 80% of patients who responded to medical treatment of a first attack of UC, had a second attack within 12 months.

The efficacy of SASP as maintenance treatment up to 5 years was subsequently confirmed by Dissanayake and Truelove.[7] The prevention of relapse in SASP group was found to be 12% vs 54% in the placebo group ($p<0.001$). It was concluded for the first time that maintenance treatment of UC with SASP should be continued "indefinitely", unless contraindicated by side effects. The importance of these observations was really relevant from a clinical point of view as controlled trial had shown that corticosteroids were totally ineffective in reducing the number of relapses.[8,9]

SASP became therefore the drug of choice of UC over the world, but the mechanism of action was still unknown.

An additional side effect was observed, consisting of reversible, no dose-related oligospermia.[10]

3. Pharmacokinetics of SASP

In the early seventies studies on the distribution of SASP and its metabolites showed that most of the SASP reached the colon intact and here it was split by bacteria azo-reductase releasing SP and 5-ASA. It was also seen that most of 5-ASA was eliminated in the faeces.[11] The SP was absorbed into circulation and was thought to be responsible for many of the adverse events of SASP. These observations were confirmed by Das who showed that in patients after colectomy the absorption of SASP was normal, while that of SP was quite absent.[12] Which of these components was the active moiety was however still unknown.

A great contribution to the history of salicylates came from Azad Khan and Truelove who provided the elegant demonstration that 5-ASA was the therapeutic moiety of SASP. Enemas of SASP, SP and 5-ASA were blindly administered to patients with active UC. Improvement was observed in approximately 30% of the patients receiving SASP or 5-ASA, and in only 5% of those receiving SP.[13] The majority of SP is absorbed from the colon and transported to the liver, where genetically determined acetylation occurs.[14] Slow acetylators have higher serum level of free SASP and are more prone to experience adverse effects as they take longer time to eliminate the drug.[15] A substantial percentage of 5-ASA is absorbed by the colon and metabolized in the epithelium to inactive acetylated form (N-Ac-5-ASA).

Two important issues were established: 1) SP acts merely as a carrier which ensures that 5-ASA is released within the colon and 2) 5-ASA could be given through rectal administration. The problem of how to take 5-ASA in the colon by oral administration without the use of SP was rapidly resolved by the realization of several delivery systems. At this point of the history, studies were mainly addressed to the mechanism of action of 5-ASA.

4. Mechanisms of action of 5-ASA

The mechanisms of action of 5-ASA are numerous and not entirely understood. It has a potent inhibitory effect on a number of pro-inflammatory mediators released by the mucosa, including Roms, Leukotrienes, Interleukin 1 and Tumour Necrosis Factor alpha (TNF α)[16–18] (Fig. 2). Recently it has been shown that the peroxisome proliferator activated receptor γ (PPARγ) is the major functional receptor mediating the common salicylate activities in IBD.[19] PPAR γ is a nuclear receptor which plays a central role in the regulation of inflammatory signalling pathways by inhibiting mucosa production of inflammatory cytokines. Very recent studies have demonstrated that 5-ASA is a ligand for PPAR γ and acts as a PPAR γ agonist.[20]

The action of 5-ASA is predominantly topical at the site of inflammation. Studies performed by our group showed that

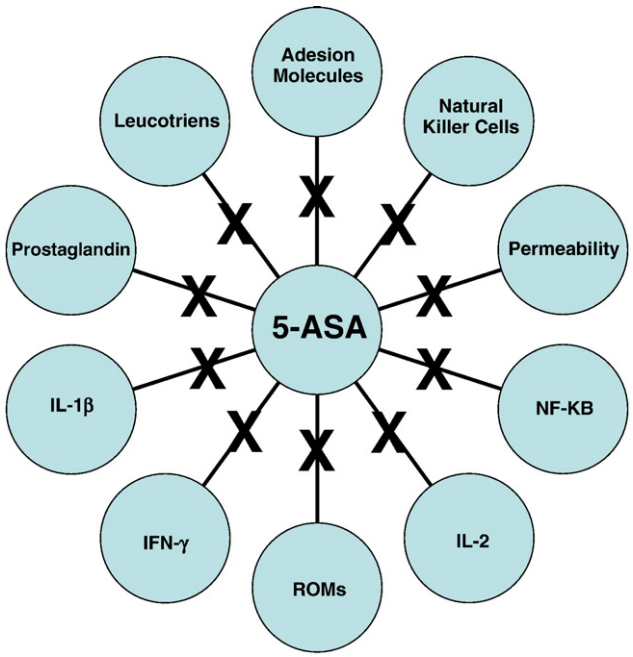

Figure 2 Mechanisms of action of 5-ASA. Mesalazine inhibits many inflammatory mediators.

the anti-inflammatory effect of mesalazine was closely correlated to its mucosal concentration.[21,22] More in particular, mucosal concentration of 5-ASA was significantly higher in patients with lower histological inflammation compared with those with more severe inflammation. A significant inverse correlation was found between 5-ASA and sIL-2R mucosal concentrations (Fig. 3). The clinical goal is therefore to maximize the delivery of 5-ASA to the colonic affected mucosa. To this purpose, the drug may be either instilled directly into the rectum and distal colon by suppositories, enemas or foam or given by mouth with different systems that assure a colonic drug delivery. In fact, administered as free 5-ASA, the drug is rapidly absorbed in the proximal small bowel and is ineffective. To obtain the best therapeutic results, therefore, it is not important to increase the oral dose, but assure a topical availability of the drug onto the inflamed mucosa. In fact it has been demonstrated that increasing the doses of oral mesalazine did not result in higher remission rate in UC.[23,24]

To summarize, oral formulations are better accepted by the patients but allow adequate mucosal concentration only in the right colon.[25] On the other hand, rectal administration allows adequate concentration in the rectum and left colon but is not always well accepted by the patients (leakage, problem with retention, burning sensation and bloating).

5. Rectal 5-ASA administration

Several RCTs have demonstrated that in UC rectally administered 5-ASA is superior to placebo, rectally administered steroids and oral 5-ASA for the induction of symptomatic, endoscopic and histological improvement and remission.[26–30]

A meta-analysis on 7 trials performed to evaluate treatments in the management of active distal UC showed that rectal 5-ASA is significantly superior to rectal corticosteroids in inducing remission of symptoms, endoscopy and histology. The pooled odds ratios (POR) were: 2.42 (95% CI 1.72–3.41), 1.89 (95% CI 1.29–2.76), and 2.03 (95% CI 1.28–3.20), respectively. The POR vs placebo were 7.71, 6.55, and 6.91 respectively.[31]

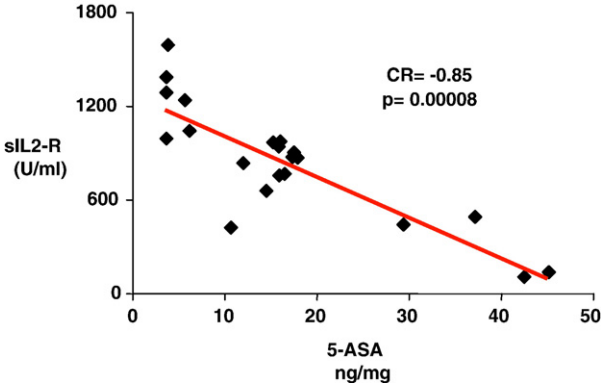

Figure 3 Chart showing the inverse correlation between mucosal concentrations of 5-ASA and sIL-2R. sIL-2R is considered a marker of mucosal inflammation [adapted from Ref. 22].

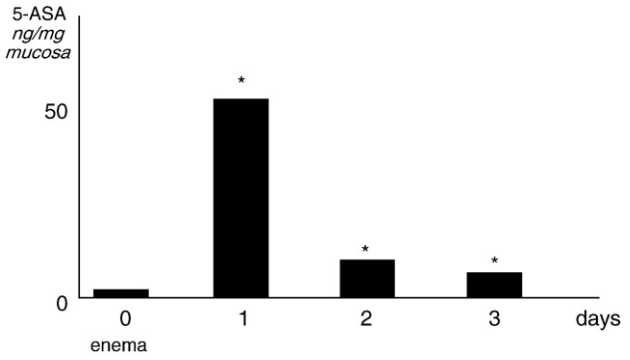

Figure 4 Rectal mucosal concentration of 5-ASA obtained 1, 2 and 3 days after a 4 g 5-ASA enema. Time 0 represents the mean mucosal concentration obtained by previous chronic oral treatment alone. Details in the text.

In addition several RCTs demonstrated the superiority of rectal 5-ASA compared to oral 5-ASA in the treatment of left-sided UC,[32–34] indicating that the rectal route is the most effective in this form of UC. Recently, 5-ASA at high dose continuously given by rectum, was able to modify also the clinical course of frequently recurrent, severe form of UC.[35] Mucosal pharmacokinetics data from our group showed that after a 4 g enema of 5-ASA given to a patient on oral treatment alone (day 0), mucosa drug concentration in the rectal mucosa was significantly higher in day 1 (24 h after rectal instillation) with respect to day 2 ($p<0.0003$), day 3 ($p<0.000007$) and day 0 ($p<0.000001$). Day 2 did not differentiate from day 3, but was statistically different from day 0 ($p<0.0001$). Day 3 was statistically different from day 0 ($p<0.001$) (Fig. 4). These data mean that topical treatment may give a therapeutic gain even if administered every three days.

6. Oral 5-ASA administration

Due to its topical action rectal 5-ASA is of limited value in UC proximal to splenic flexure. Thus, many studies were performed in order to develop new delivery systems able to release the drug in the colon. Currently 4 types of formulations are available:

1. Compounds that bind 5-ASA to a carrier requiring splitting of the diazo-bond by bacteria (Olsalazine and Balsalazide);
2. Compounds pH dependent (Asacol);
3. Time-controlled release microsphere (Pentasa);
4. Multi-matrix (MMX) system (Lialda).

The first idea of synthesizing a compound able to deliver 5-ASA to the colon without the side effects of SP came from Truelove who carried out a new compound called disodium azodisalycilate (Olsalazine) consisting of two salicylate radicals linked by an azo-bond.[36] The drug was largely studied by the Oxford group and resulted to be effective in UC but its use was limited by frequent occurrence of diarrhoea.[37]

At the same time the group of Lennard-Jones synthesized a pro-drug consisting of an inert compound 4-Aminobenzoy-lalanine linked to 5-ASA (Balsalazide).[38] This drug after preliminary encouraging results fell into disuse.

The pH-dependent 5-ASA formulations were introduced by John Rhodes, from Cardiff, who found a gastro-resistant acrylic resin, Eudragit-s that dissolves in alkaline medium and is therefore able to transport into a capsule 5-ASA to the terminal ileum and caecum where it is delivered (Asacol).[39]

Another oral 5-ASA preparation consists of 5-ASA coated with microsphere of ethyl-cellulose that allows slow release of the medication beginning in the duodenum and extending into the proximal colon (Pentasa).[40]

The different mesalazine formulations commercially available show different profiles of release (Fig. 5). The limitations of these delivery systems are the frequent daily dosing together with the number of pills required per day that reduce patient compliance with long term therapy. In fact non-adherence to therapy is a widespread problem in chronic diseases. An estimated 20% to 50% patients with UC do not take medications as prescribed, resulting in higher relapse rates with consequent higher health-care costs and, possibly, greater risk of CRC.

According to ECCO consensus,[41] currently most of the patients with mild–moderate UC are treated by oral formulations of 5-ASA both for induction and maintenance of remission. Two Cochrane reviews have been recently published on the effectiveness of oral 5-ASA for induction and maintenance of remission in UC. 5-ASA was compared to placebo or SASP. The conclusions of these reviews were that oral 5-ASA preparations were superior to placebo and tended toward therapeutic benefit over SASP for induction of remission.[42] However as far as maintenance therapy, 5-ASA preparations were superior to placebo, but had a statistically significant therapeutic inferiority relative to SASP.[43]

These results are very important from a practice point of view as oral 5-ASA formulations are the first-line therapy commonly used for the maintenance of remission in UC. The consequences of this strategy on health-care costs are relevant. It has been recently shown that the costs of oral 5-ASA products are the major component of the long term medicative expenditure in IBD.[44] In particular it has been estimated that the cost of 5-ASA 2.4 g/day is 198 $ monthly, while that of SASP is 15 $ monthly.[45] Considering that SASP yields comparable or even better benefits of 5-ASA and that 5-ASA is more than 10 folds expensive, the maintenance treatment with SASP is cost-effective and, if tolerated, could be recommended.

7. New oral mesalazine formulation

Recently a new oral 5-ASA formulation, the MMX system, was licensed by Giuliani S.p.A., Milan Italy with the purpose of improving compliance and delivering the 5-ASA throughout the colon more distally with respect to the previous formulations.[46,47] This formulation contains a double matrix system that uses a lipophilic matrix dispersed within a hydrophilic matrix. The tablet core contains microparticles of 5-ASA in the lipophilic matrix, which are dispersed through the hydrophilic matrix.

The core is then coated with a pH-dependent, gastro-resistant polymeric film that is designed to allow the coating to disintegrate at pH $\geq$ 7.0. This coating matrix and coating system is designed to begin dissolution in the terminal ileum. Here the hydrophilic matrix begins to erode and 5-ASA diffuses out of the lipophilic matrix. The MMX delivery system allows the homogeneous distribution of 5-ASA through the ascending, transverse, descending, sigmoid colon and rectum.[46]

Among the oral formulation of 5-ASA Asacol and Pentasa have been largely investigated by RCTs and represent more than 90% of the salicylate market. MMX mesalazine, to date is commercially available only in the US as 1.2 g tablets (Lialda, Shire, Pharmaceuticals Inc., Wayne, PA). This high dose formulation should improve compliance. The once-daily MMX tablet, has been developed with the aim of both increasing the adherence to oral mesalazine treatment and avoiding the topical administration of the drug. The efficacy of MMX mesalazine (1.2 g) was compared with mesalazine enema (4 g) in patients with left-sided active UC. Clinical remission occurred in 60% of pts in MMX group and in 50% of enema group. Similar improvement was seen in the endoscopic and histological pattern. In addition the adherence rate in remission was 92% in MMX group and 65% in enema group.[47] Recently two RCTs showed that once-daily MMX mesalazine was efficacious and well tolerated in active mild to moderate UC for induction of clinical and endoscopic remission.[48,49] No

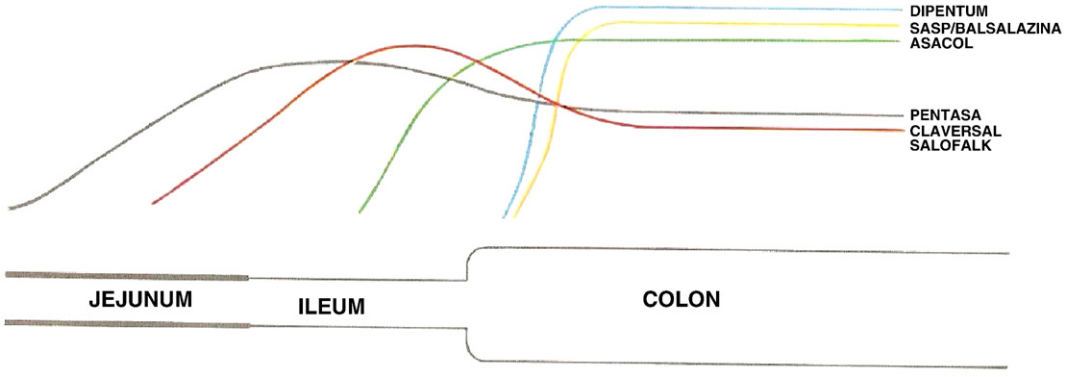

Figure 5 Release profiles of various commercially available formulations of oral mesalazine. Pentasa, Claversal and Salofalk mostly deliver in the small bowel, whereas Dipentum and SASP in the colon.

significant difference in remission rate was found between the MMX 2.4 g/day group and MMX 4.8 g/day group.[49] Safety data appear similar to those of other 5-ASA. No serious adverse events were reported.

Conclusions arising from these studies are that MMX mesalazine offers effective and convenient 5-ASA therapy also for distal UC and improves treatment compliance allowing once- or twice-daily administration.

8. New targets of salicylates

Several observational studies have reported the effectiveness of the long term administration of both SASP and mesalazine in the prevention of colorectal cancer (CRC) in UC.[50,51] Other studies failed to show chemopreventive effect of mesalazine.[52,53]

These studies however are mainly retrospective and include not very large UC populations. A meta-analysis including 9 studies allowed to reach a total population of 1.932 UC subjects and showed a protective association between the use of 5-ASA and CRC (OR=0.51; 95% CI 0.37–0.69).[54]

These data are also in keeping with the results of large epidemiological studies that demonstrated that continue use of FANS, in particular aspirin, compounds chemically related to 5-ASA are effective in preventing development of CRC in the general population. The mechanisms of chemoprevention are not completely understood but the control of inflammation, a well known trigger in the pathway of colitis–dysplasia–carcinoma sequence, has been advocated. 5-ASA, in fact, decreases cell turn-over (by promoting apoptosis through COX-dependent pathways), acts as antioxidant and free radical scavenger reducing DNA oxidative stress and microsatellite instability.[55] Recently it has provided evidence supporting the role of activation-induced cytidine deaminase (AID) as a link between inflammation and UC associated CRC.[56]

However, some mechanisms other than control of inflammation should be taken under consideration as the use of immunosuppressive drugs seems not to reduce cancer risk.[57] In fact many observations suggest that 5-ASA can reduce the risk of cancer by interfering directly with carcinogenetic cell biology, other than by simply controlling inflammation. Many other molecular mechanisms have been advocated for the anti-neoplastic activity of mesalazine.[53] Firstly the direct inhibition of COX-2 by 5-ASA leading to the reduction of many prostaglandins, including PGE2, that sustains various functions of tumour cells, including proliferation, survival, angiogenesis and invasion.

Another anti-neoplastic effect of 5-ASA relies on its ability to inactivate protein phosphatase 2A (PP2A) and the epidermal growth factor receptor (EGFR) that play key roles for the development of cancer. Moreover, 5-ASA PPAR-γ and experimental models demonstrated the direct anti-neoplastic effects of 5-ASA due to its selective affinity for PPAR-γ. Recently it has been shown that 5-ASA is a ligand for PPAR-γ in colonic epithelial cells[19,20] and functions as a PPAR-γ agonist. Moreover NF-kappaB activation has been demonstrated to be the key to inflammation-associated colon cancer.[58] Mesalazine, therefore, may display its anti-cancer effect also by blocking the epithelial activation of NF kappa B itself. This may pave the way to the preventive role of mesalazine even for sporadic colon cancer. In conclusion a lot of evidence indicate that 5-ASA interfered with many inflammatory pathways involved in the initiation and progress of CRC. In addition, it has also been recently shown that 5-ASA may directly affect CRC cells interfering with their growth and survival in particular blocking growth and promoting apoptosis.[51] However it is important to consider that in vitro studies demonstrated that the anti-neoplastic effect of 5-ASA is present only with relative high drug concentrations (10–50 mmol/L), which are not always reached within the colonic tissue under standard oral treatment. Therefore, although several molecular mechanisms underlie the anti-neoplastic action of mesalazine, further prospective clinical studies are needed before its use may be recommended in clinical practice as chemoprevention of CRC in UC.

9. Conclusive remarks

Seventy years after Nanna Svartz's discovery of SASP, salicylates still play a central role in the treatment of UC. The scarce side effects, the relative low cost and the effectiveness make these drugs still competitive even in the era of biological agents. The new oral formulations, improving patient compliance and allowing treatment of left-sided colitis, may open new roads to an old drug.

Conflict of interest

Prof. Renzo Caprilli is involved in a trial on mesalazine MMX sponsored by Giuliani S.p.A. (Milan, Italy). Giuseppe Frieri, MD, Erika Angelucci, MD, and Monica Cesarini, MD, have no conflict of interest.

Acknowledgment

The authors wish to thank Mrs. Maria Elena Farese for help in reviewing the English language of the manuscript.

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