PROTECTIVE EFFECTS OF SOME AZO DERIVATIVES OF 5-AMINOSALICYLIC ACID AND THEIR PEGYLATED PRODRUGS ON ACETIC ACID-INDUCED RAT COLITIS

[1]ALIREZA GARJANI, [2]SOODABEH DAVARAN, [2]MOHAMADREZA RASHIDI AND [1]NASRIN MALEKI

[1] Department of Pharmacology, School of Pharmacy, [2] Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

ABSTRACT

The protective and anti-inflammatory effects of azo and azo-linked polymeric prodrugs of 5-aminosalicylic acid (5-ASA) on acetic acid induced colitis in rats were investigated. Three azo prodrugs; 4,4'-dihydroxy-azobenzene-3-carboxilic acid (azo compound I), 4-hydroxy-azobenzene-3,4'-dicarboxilic acid (azo compound II), 4,4'-dihydroxy-3'-formyl-azobenzene-3-carboxylic acid (azo compound III) and their polyethylene glycol (PEG 6000) derivatives were synthesized. Rats were pretreated orally (1 hour prior to induction of colitis) with sulfasalazin (300 mg/kg), azo compounds I, II, III and polyethylene glycol conjugates of azo compounds II and III in doses which had the same amount of 5-ASA as sulfasalazin contains. The colonic damage was examined 24 hours later and characterized by gross microscopic injury and colonic edema. Among prodrugs only azo compound III (215 mg/kg) produced a significant ($p<0.01$) protective effect against colonic injury comparable with sulfasalazin. Doubling the dose (430 mg/kg) showed more anti-colitis effects. Polyethylene glycol conjugate of azo compounds II and III also showed reduction in the extent of the cell death and tissue disorganization similar to sulfasalazin. While neither sulfasalazin, nor azo compound II and its PEG polymer produced anti-edema effects, both azo compound III and its PEG polymer decreased colon edema significantly ($p<0.05$). Histological examinations also indicated a marked reduction in tissue injury and inhibition in neutrophil infiltration in rats treated with azo compound III and PEG conjugates of azo compounds II and III. Results of this investigation provide experimental evidence supporting new cytoprotective, anti-inflammatory and anti-edema properties of the azo derivatives of 5-ASA and their PEGylated prodrugs.

Key words: Azo linked polymer; Azo prodrug; 5-ASA; Acetic acid; Colitis; Anti-edema

INTRODUCTION

There is a considerable interest in the colon specific drug delivery in order to treat diseases of the large intestine, such as colitis, colon cancer, irritable bowel syndrome, Crohn and infectious diseases (1-6). Colon targeting may be achieved by different delivery systems. The discovery that the active moiety in sulfasalazin, 5-aminosalicylic acid (5-ASA), is generated by the action of the intestinal microflora has been the trigger for colon-specific drug delivery through enzymatic reduction of the azo bond (7-10). Colon is known to be a reductive medium in which azo groups are reduced to the corresponding amines (11-14). It has been shown that polymeric azo compounds could be used for colon targeting since reduction and subsequent splitting of the azo bond occurs only in the large intestine, and therefore they are highly site-specific (15-17). This opportunity for reductive degradation of azo compounds by microflora of colon has been exploited to prepare low molecular or macromolecular prodrugs of the anti-inflammatory agent 5-ASA (18-25). In this study, some new azo derivatives of 5-ASA and related PEGylated prodrugs were synthesized and their anti-inflammatory and cytoprotective effects were evaluated in an in vivo model of the colonic inflammation in rats in which damages were induced by the intrarectal administration of acetic acid.

MATERIALS AND METHODES

Materials

The monomers and polymers were characterized by the IR and NMR spectroscopy. IR spectra were recorded by the use of KBr pellets on a Shimadzu 4300 spectrophotometer. ^{1}H NMR spectra of PEGylated prodrugs in DMSO (d6) were recorded on the 400 AM Bruker Spectrometer. 5-ASA was purchased from Aldrich and recrystallized from water and ethanol, respectively. All other reagents were obtained from Merck Chem. Co.

Correspondence: Alireza Garjani, Department of Pharmacology, School of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran, E-mail: garjania@tbzmed.ac.ir

Methods

Synthesis of 4,4'-dihydroxy-azobenzene-3-carboxylic acid (azo compound I)

A solution of 9.66 g (0.07 mol) of salicylic acid and 23 g of sodium hydroxide in 250 ml H_2O was added to a diazonium salt solution prepared from 5.33 g (0.048 mol) ρ-aminophenol and 3.6 g sodium nitrite (3-5°C). The mixture was kept for 24 hours at room temperature and acidified with concentrated HCl. The precipitate was filtered off, washed with H_2O, dried and dissolved in the hot solution of ethanol (20 ml) and acetone (5 ml). The hot mixture was filtered and the solution was concentrated to 60 ml. After addition of 20 ml of H_2O and cooling the solution, the precipitate was separated and washed with 40 ml of methanol and recrystalized from toluene. The crystals obtained were dried in an oven at 120°C to yield 11 g (61%) 4,4'-dihydroxyazobenzene-3-carboxilic acid (scheme 1) crystals with orange color and melting point of 159°C. IR (KBr), ν (cm^{-1}): 895 (aromatic C-H), 1160 (C-O), 1200-7300 (C-N), 1450-1550 (aromatic C=C), 1660 (C=O), 3020 (aromatic C-H), 3240 (phenolic OH), 2500-3200 (acidic O-H).

^{1}H NMR (DSMO) δ (ppm): 11 (1H, COOH), 8 (1H, orto to COOH), 7.8 (1H, para to COOH), 6.9 (1H, meta to COOH).

Synthesis of 4-hydroxy-azobenzene-3,4'-dicarbo-xilic acid (azo compound II)

The titled compound was prepared by coupling of salicylic acid (9.44g, 0.07mol) with diazonium salt of ρ-aminobenzoic acid according to the method described above. The compound was obtained in 68.5% (14.2g) yield as yellow crystals with melting point of 223°C. Azo compound II:

IR (KBr), ν (cm^{-1}): 895 (aromatic C-H), 1160 (C-O), 1200-7300 (C-N), 1450-1550 (aromatic C=C), 1660 (C=O), 3020 (aromatic C-H), 3240 (phenolic OH), 2500-3200 (acidic O-H).

^{1}H NMR (DSMO) δ (ppm): 10.8 (2H, COOH), 8.3 (3H, orto to COOH), 7.8 (1H, para to COOH), 6.8 (3H, meta to COOH).

Synthesis of 4,4'-dihydroxy-3'-formyl-azobenzene-3-carboxylic acid (azo compound III)

5-Amino salicylic acid 0.048 mol (7.34g) was dissolved in a mixture of 5 ml concentrated HCl and 15 ml of water by heating and insoluble impurities were filtered off. Crushed ice (25g) and 7.5 ml of concentrated HCl were added to the resulting solution and the mixture was stirred continuously. When the temperature reached to about 3°C a cold solution of sodium nitrite (3.6g; 0.05 mol) in 7 ml H_2O was added and then salicylic aldehyde (8.54 g; 0.07 mol) and NaOH (20 ml of 5% solution) were added to the resulting solution of the diazonium salt. The mixture was stirred for 15 min at about 3°C and kept at room temperature overnight.

The compound was obtained in the form of sodium salt (pH 9.5-10), which was precipitated by addition of concentrated HCl (pH 1-2). The solid compound was dissolved in 20 ml hot ethanol containing 5 ml acetone. The solution was then filtered; concentrated and cooled. The precipitate was collected, recrystalized from acetic acid-water (50:50) and dried to yield 7.73 g (54.5%) of azo compound (brown crystals) with melting point of 117-120°C. Azo compound III:

IR (KBr), ν (cm^{-1}): 1200-1300 (C-N), 1450-1490 (aromatic C=C), 1670 (C=O), 2750-2850 (aldehyde C-H), 3070 (aromatic C-H), 2500-3200(acidic OH).

^{1}H NMR (DSMO) δ (ppm): 10.5-11 (1H, COOH), 10.2 (1H, CHO), 8.1 (2H, orto to COOH and CHO), 7.8 (2H, para to COOH and CHO), 7.1 (2H, meta to COOH and CHO).

General procedure for the preparation of polyethylene glycol derivatives of azo compounds

9 grams of Polyethylene glycol (PEG 6000, 1.5 mmol), 0.22 grams of 4-(N, N- dimethylamino) pyridine (DAMP, 1.8 mmol), and 0.37 grams of N, N-dicyclohexyl carbodiimide (DCC, 1.8 mmol) were stirred in 20 ml DMF at room temperature for 30 min. The corresponding azo derivatives of 5-ASA (1.8 mmol) were added to the mixture and the mixture was then stirred for 6 hours at room temperature. The white precipitates of dicyclohexyl urea (DCU) were filtered and the polymer was purified by repeated crystallization from a mixture of diethyl ether and methylene chloride (1:1) to yield the respective polymer-drug conjugated (PEG-azo compounds I, II and III). IR (KBr), ν (cm^{-1}): 1450-1550 (aromatic C=C), 1710 (ester carbonyl), 1010-1025 (C-O), 3240 (OH phenolic).

Induction of acetic acid colitis

Male wistar rats (230-260g), obtained from the laboratory Animal Services of the Medical Sciences University of Tabriz, were randomly distributed in 9 experimental groups as shown in table 2. Animals were housed in cages (5 rats per cage) and kept in the laboratory in air-conditioned animal quarters with a 12-h light-dark cycle and had free access to tap water and food for 1 week before the beginning of experiments. The induction of acetic acid colitis was adapted by the method described by Myers and coworkers (26). Drugs or carrier were administered per orally by using a gavage as a suspension or solution in 1 ml tap water 1 hour before induction of colitis.

Animals were fasted for 24 hours and anaesthetized slightly with diethyl ether. Under anaesthesia, colitis was induced by an enema of 4% (v/v) acetic acid. The solution was delivered by means of a Teflon cannula (o.d.2mm) inserted 7cm through the anus. The enema was expelled after 20 seconds of contact with the colonic mucosa by gentle massage of the abdomen. When the administration was completed, animals were kept in a head-down position until they recovered from anesthesia, and then were returned to their cages.

Assessment of colonic damage

Twenty four hours after induction of colitis, animals were sacrificed and their colons were excised for the assessment of damages. The colonic segments were placed on an ice-cold plate, cleaned from fat and mesentery, and blotted on filter paper. Each specimen was weighed and its length was measured under a constant load (2g). To account the extent and the severity of the colonic damages, the colon was longitudinally opened and scored for macroscopically visible damages from zero to 10 scales (table 1) by two observers unaware of the treatment (27). Three rats in each group randomly selected for histological determination.

Assessment of edema

In all animals, the ratio of wet tissue weight to length of the colon was estimated in order to evaluate the intensity of the edema (27).

Histological examination

For histopathological study, biopsies of normal and damaged tissues (n=3) of all groups were obtained from colon and fixed in 10% neutral-buffered formaldehyde, embedded in paraffin and sectioned. The sections were stained with haematoxylin and eosin.

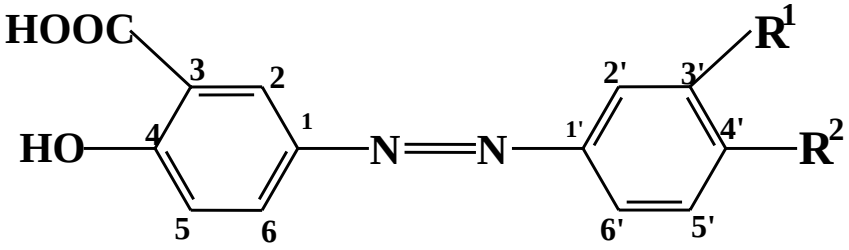
Statistical analysis

All results are expressed as mean ± SEM. Differences among groups were tested for statistical significance using unpaired student t test (two tailed p value). A probability of $p< 0.05$ was considered statistically significant.

RESULTS

The intrarectal instillation of acetic acid in rats led to development of diffuse changes in the colon consisting of erythema, inflammation and confluent areas of extensive erosions and hemorrhage extending approximately 2cm in length in average. They were assigned a mean score of 5.2±0.4. In this study nine groups of animals were studied. All animals received a single dose of drugs orally, 1 hour prior to induction of colitis. All selected doses of azo compounds in this study contained the same amount of 5-ASA as sulfasalazin contains. The first group that received saline considered as control group. In the second group which received PEG 6000 (1.24 g/kg) the ulcerative lesions were similar to the control group, showing that PEG as a polymer constituent of the azo polymeric compounds had no effects on the acetic acid induced colitis and edema (table 2). The scoring index in this group was 5.2±0.2. Compared to the control group, treatment of colitic animals with sulfasalazine (300mg/kg) as a reference drug had no effect on the tissue edema, but it significantly reduced the damage score from 5.2±0.4 in the control to 2.6±0.7 ($p<0.01$). Majority of the rats treated with sulfasalazine presented a site of gross ulceration and inflammation, not extending more than one centimeter along the colon. Treatment with neither azo compound I (200mg/kg) nor azo compound II (220 mg/kg) had protective effects against the colonic inflammation and edema. However, similar to sulfasalazine, PEG polymer of azo compound II (1.4 g/kg) reduced the damage score significantly ($p<0.01$) to 2.6±0.7 compared to control (AA) without any significant effects on the tissue edema (table 2). In contrast to the azo compounds I and II, azo compound III in which salicyl aldehyde is part of the molecule, caused marked reduction of both colotic inflammation and tissue edema (table 2). A dose of 215 mg/kg of azo compound III decreased the damage score to 2.6±0.5 ($p<0.01$) and the edema to 140.5±7.2 mg/cm ($p<0.05$). The majority of rats received azo compound III showed no ulcer and only moderate petechia and hypervascularity were observed in colons. The anti colitis effect of azo compound III was comparable to that of sulfasalazine with scoring index of 2.6±0.7, whereas sulfasalazin as a reference drug had no anti-edema effect. Increasing the dose of azo compound III to 430 mg/kg produced more protective effect against macroscopical colonic damage (score: 1.4±0.2; $p<0.01$) and edema (119±3.9 mg/cm; $p<0.01$). Polymerization of azo compound III with PEG created no further anti-colitic and anti-edema compared to non-polymeric azo compound III and sulfasalazin. In this group similar to sulfasalazin and azo compound III treated animals, the damage score and edema were reduced to 2.8±0.4 and 140±6.8 mg/cm ($p<0.05$), respectively.

The histological sections obtained from colon of PEG treated rats and animals from the control group showed pethechia, hypervascularity, submucosa edema, epithelial disruption, mucosal erosion with goblet cell depletion and PMN



Scheme 1. Azo compounds prepared. I: R_1=H, R_2=OH; II: R_1=H, R_2=COOH; III: R_1=CHO, R_2=OH.

Table 1: Details of the grading system for estimation of the severity of mucosal inflammation using a scoring index.

Score	Grouping	Macroscopic Appearance
0	No inflammation	Normal
1	Mild inflammation	No ulcer, mild petechia/hypervascularity
2	Mild inflammation	No ulcer, moderate petechia/hypervascularity
3	Moderate inflammation	Ulcer<1 cm with petechia/hypervascularity
4	Moderate inflammation	Same as above at 2 or more sites
5	Moderate inflammation	Ulcer 2 cm with petechia/hypervascularity
6	Severe inflammation	Ulcer 3 cm with petechia/hypervascularity
7	Severe inflammation	Ulcer 4 cm with petechia/hypervascularity
8	Severe inflammation	Ulcer 5 cm with petechia/hypervascularity
9	Severe inflammation	Ulcer 6 cm with petechia/hypervascularity
10	Severe inflammation	Ulcer >6 cm with petechia/hypervascularity

Table 2: Effects of azo derivatives of 5-amino salicylic acid and their PEGylated prodrugs on the damage score and changes in colonic weight 24h after acetic acid (AA) administration

Group	N	Damage score (0-10)	Colonic weight (mg/cm)
AA Control	9	5.2±0.4	165.2±4.7
PEG (1.24 g/kg)	5	5.2±0.2	174.5±4.7
Sulfasalazine (300 mg/kg)	5	2.6±0.7**	169.2±15.7
Azo I (200 mg/kg)	5	5.0±0.5	170.4±9.7
Azo II (220 mg/kg)	5	4.4±0.8	165.4±9.9
Azo III (215 mg/kg)	5	2.6±0.5**	140.5±7.2*
Azo III (430 mg/kg)	5	1.4±0.2**	119.0±3.9**
PEG-Azo II (1.4 g/kg)	5	2.6±0.7**	158.6±11.9
PEG-Azo III (2.4 g/kg)	5	2.8±0.8*	140.0±6.8*

*p<0.05, **p<0.01 vs. acetic acid (AA) control group

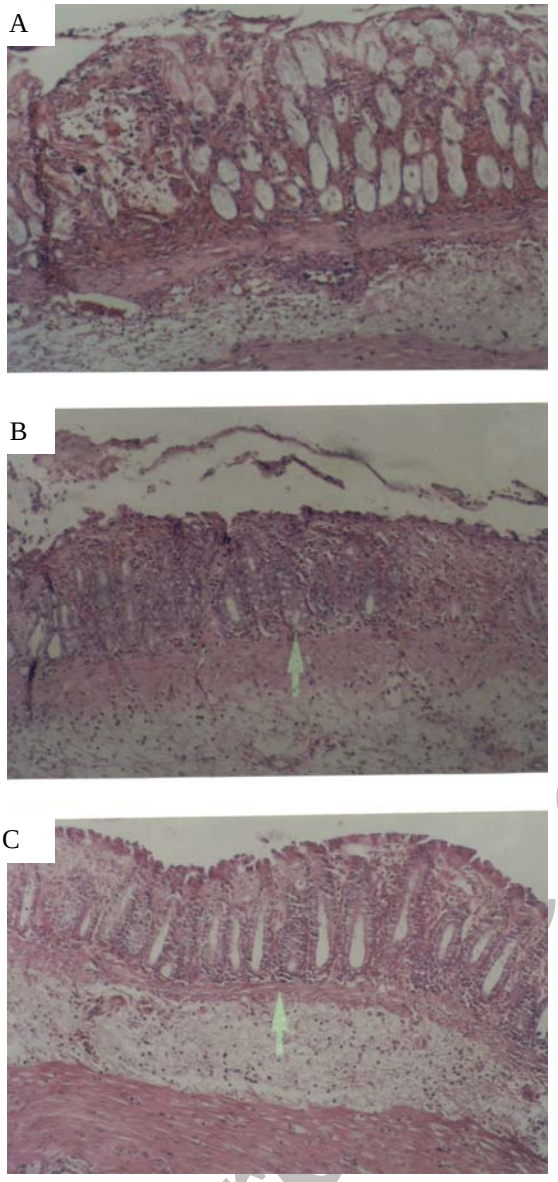


Figure 1. A colon slice from a colitis-induced rat (A), showing submucosa edema, epithelial distruption and mucosal erosion with goblet cell depletion. The tissue section from a colitis-induced rat that received sulfasalazin (B) and azo compound III (C) demonstrated reduced oedematasis and PMN infiltration. Arrows indicate the basal layer of the epidermis. M×10

infiltration (Fig 1a). As shown in Fig 1b, in sulfasalazin treated group the histological changes were minimized and mucosal glands were intact, but there were slight hyperemia, goblet cell reduction and considerable submucosal edema. The PMN infiltration was also reduced in this group. The microscopic study of the tissue slices from azo compound III treated animals revealed complete healing of mucosa with no hyperemia and no signs of epithelial sloughing and goblet cell depletion. In this group there was considerable reduction in PMN infiltration and submucosal edema (fig 1C).

DISCUSSION

Several diseases such as inflammatory bowel syndrome (IBS) can be treated more effectively by local delivery of anti-inflammatory agents to the colon. It is well known that sulfasalazin and its metabolite 5-aminosalicylic acid are anti-inflammatory drugs which are used in the treatment of ulcerative colitis. In addition to the limitation of the rate and amount of release of 5-ASA from sulfasalazin, sulfapyridine moiety may leads to some side effects. Reduction and hydrolysis are the predominant process in the colon. In this sense, different types of polymeric and non-polymeric azo-prodrugs containing 5-ASA can be evaluated for colon-specific drug delivery. The azo-reductase activity of colonic bacteria has been used to liberate the active agents in the colon from azo- compounds. Therefore, the release and absorption of the active agents in colon would protect the drugs from gastric enviroment. In this respect, three novel azo prodrugs of 5-ASA and their PEG derivatives were synthesized.

Previously, the preparation and hydrolytic properties of polymethacrylic acid (PMAA)-PEG complexes conjugated with 5-ASA in which 5-ASA residues were attached to the PEG chains via ester bonds was described (20-21). The results obtained from the hydrolysis of PMAA-PEG conjugates of 5-ASA indicated that a small amount of 5-ASA was released as the pH of hydrolysis medium increased. Taking into account that there is no sufficient pH gradient between small and large intestine (e.g. ileum and cecum), a certain amount of the drug can be released by hydrolysis of the swollen polymer in small intestine. In order to overcome this problem, in the present study the 5-ASA was converted to its azo derivatives prior to attachment to polymeric carrier and as a result water-soluble polymeric prodrugs were obtained in which azo derivative of 5-ASA was linked to the end group of PEG through an ester bond. The azo derivative can act as a second low-molecular weight carrier after partial hydrolysis of the ester bond in the upper regions of the intestine.

Instillation of 4% acetic acid into the rat colon caused an intense acute inflammatory response, which was indicated by macroscopical and microscopical damages of the colonic wall, ulcer, hyperemia, hypervascularity, submoucosal edema and depletion of goblet cells. In this study, sulfasalazin was used as a comparative control.

Azo compounds II and I had no effect on the tissue damage and edema. However, administration of azo compound III prior to colitis inductions at a dose containing an equal amount of 5-ASA that sulfasalazin contains prevented colonic inflammation similar to sulfasalazin. Doubling the dose of azo compound III produced more protective effect against the severity of the inflammatory lesions and reduced the damaged area markedly ($p<0.01$). These effects were consistent with the observation of grossly normal epithelium in histological sections obtained from rats treated with either doses of azo compound III.

One of the important and pathological features of colonic inflammation is tissue edema. Interestingly, in contrast to sulfasalazin, azo compound III ameliorated colonic tissue edema significantly ($p<0.05$). This reduction was more considerable by doubling the dose ($p<0.01$). The anti-edema effect of azo compound III may be considered as an advantage for this new azo prodrug of 5-ASA. Sulfasalazin and 5-ASA have been shown to inhibit a number of cell-mediated immune processes in vitro, such as T-cell proliferation (28, 29) and antibody secretion (30). Both sulfasalazin and 5-ASA produce inhibitory effects on macrophage and neutrophil functions by impairment of chemotaxis and adhesion (29). This study also showed reduced PMN infiltration in sulfasalazin, azo compound III and PEG-azo compound III treated rats. Sulfasalazin and 5-ASA block the production of prostaglandins (28) and tumor necrosis factor-α (31, 32) and also reduce nuclear factor κB (NF-κB; 29). The ability of azo compound III and its PEGylated derivative to produce the same anti-inflammatory action as sulfasalazin, indicates the possibility of the involvement of similar mechanisms. Azo compound III in addition to amino salicylic acide moiety contains salicylic aldehyde, which may produce more anti-inflammatory and anti-edema actions. However, to understand the exact mechanisms of the anti-inflammatory and anti-edema effects of azo derivatives more studies are required. Another approach that can be used in colon-specific delivery system is to attach a drug via an azo bond to a polymeric carrier. In this study incorporation of PEG 6000 into the azo compounds II and III gave PEGylated prodrugs of PEG-azo compound II and PEG-azo compound III. Both polymers at a single dose reduced damage score and histological outcomes 24 hours after acetic acid administration and were as potent as sulfasalazin or azo compound III. Similar to azo compound III, its PEG conjugate showed a significant ($p<0.05$) anti-edema effect. Neither Azo compound II nor PEG had any effect on the colonic tissue damages while PEG conjugate of azo compound II produced an effective anti-colitis action comparable to sulfasalazin.

The azo derivatives described here, are the dimmers of 5-ASA and ρ-aminobenzoic acid (azo II), ρ-aminophenol (azo I) and salicyl aldehyde (azo III) linked through an azo bond. When splited by colonic bacteria, the azo derivatives deliver 5-ASA. The results of this study indicated that replacement of sulfapyridine in sulfasalazine with other aromatic moieties such as benzoic acid (azo compound II), phenol (azo compound I) and salicyl aldehyde (azo compound III) altered the pharmacological properties of the drug (Tab. 2). It could be suggested that the structure of the aromatic moiety linked to 5-ASA is effective in the interaction of the drug with the active sites of the reductase enzymes. The higher anti-inflammatory and anti-edema activity of azo compound III compared to sulfasalazine could be related to the presence of salicyl aldehyde moiety which may has further anti-inflammatory or anti edema effects.

REFERENCES

1. Peppercorn, M.A., Goldman, P. (1972) The role of intestinal bacteria in the mechanism of Salicyl azo sulfapyridine. J. Pharmacol. Exp. Ther. 181: 555 – 562.
2. Jarnerol, G. (1989) Newer 5-amino salicylic acid based drugs in chronic inflammatory bowel disease. Drugs 37: 73-86.
3. Friend, D.R. (1991) Colon – specific drug delivery. Adv. Drug Deliv. Rew. 7: 149-199.
4. Geier, D.L., Miner, P.B., New, Jr. (1992) Therapeutic agents in the treatment of inflammatory bowel disease. Am. J. Med. 93:199 – 208.
5. Kamm, M., Senapati, A. (1992) Drug management of ulcerative colitis. Br. Med. J. 305: 35-38.
6. Crotty, B., Jewell, D.P. (1992) Drug therapy of ulcerative colitis. Br. J. Clin. Pharmacol. 34: 189-198.
7. Fuller, A.T. (1937) Is p-amino benzene sulfonamide the active agent in prontosil therapy? Lancet 1: 194.
8. Schacht, E., Geraert, A., Kenaw, E.R., Molly, K., Verstraete, W., Adriaensens, P. (1996) Polymers for colon specific drug delivery. J. Control Rel. 39: 327-338.
9. Schroder, H., Johansson, A.K. (1973) Redox potential in caecal contents of the rat and azo reduction of salicyl azo sulfpha pyridine. Xenobiotica 3: 233-246 .

10. Schroeder, H., Campbell, D.E. (1972) Absorption, metabolism and excretion of salicyl azo sulfopyridine in man. Clin. Pharmacol. Ther.13: 539-552.
11. Miyadera, T. (1975) Biological formation and reactions of hydrazo, azo and azoxy groups. In: Patai, S. (ed) The chemistry of hydraz, Azo and Azoxy compounds. Part 1 Wiley, New York, pp: 495.
12. Walker, R. (1970) Metabolism of azo group: a review of literature. Food Cosmet. Toxicol. 8: 659-676.
13. Radomski, J.L., Mellinger, T.J. (1962) Absorption, fate and excretion in rats of soluble azo dyes. J. Pharmacol. Exp. Ther. 136: 259-266.
14. Dieckhues, B. (1960) Reductive splitting of azo-dyes by bacteria. ZentralbI. Bakteriol. Parasiten Kd. 180: 244-249.
15. Dubru, D., Wright, K.L. (1975) Reduction of azo food dyes in cultures of Proteus Vulgaris. Xenobiotica 5: 563-567.
16. Hanohan, T.E., Enderlin, B.A, Parkinson, T.M. (1977) Intestinal absorption of polymeric derivatives of the food dyes sunset yellow and Tartriazine in rats. Xenobiotica 7: 765-701.
17. Brown, J.P. (1981) Reduction of polymeric azo and nitro dyes by intestinal bacteria. Appl. Environ. Microbiol. 41:1283-1286.
18. Chng, K.T., Funk, G.E., Egan, M. (1976) Azo dye reduction by anaerobes. Appl. Environ. Microbiol. 5: 558-562.
19. Lloyd, A., Martin, G.P., Soozandehfar, S.H. (1994) Azopolymers: A means of colon specific drug delivery. Int. J. Pharm. 106:255-260.
20. Davaran, S., Hanaee, J., Khosravi, A. (1999) Release of 5-amino salicylic acid from acrylic type polymeric prodrugs designated for colon specific drug delivery. J. Control Release 58: 279-287.
21. Davaran, S., Rashidi, M.R., Hashemi, M. (2001) Synthesis and characterization of methacrylic derivatives of 5-amino salicylic acid with pH-sensitive swelling properties. AAPS Pharm. Sci. Tech. 2(4): article 29.
22. Rusmussen, S.N., Brondesen, S., Hridberg, E.F., Honore, S., Binder, V., Halshov, S. (1982) 5-Aminosalycilic acid in slow-release preparation: Bioavailability, plasma level and excretion in humans. Gastroentrology 83:1062-1070.
23. Christensen, L.A, Slot, O., Sanches, G., Boserup, J., Rasmussen, S.N., Bondesen, S. (1987) Release of 5-ASA from pentasa during normal and accelerated intestinal transit time. Br. J. Clin. Pharmacol. 23: 365-369.
24. Brown, J.P., McCarraugh, G.V., Parkinson, T.M., Wingard, Jr. R.E., Onderdonk, A.B. (1983) Polymeric drug for treatment of inflammatory bowel disease. Am. J. Med. Chem. 26:1300-1307.
25. Callant, D., Schacht, E. (1990) Macromolecular prodrugs of 5-amino salicylic acid, 1:Azo conjugates. Makromol. Chem. 191: 529-536.
26. Myers, Brian, S., Martin, J.S., Dempsey, D.T. (1997) Acute experimental colitis decreases colonic circular smooth muscle contractility in rats. Am. J. Physiol. 273:G928-G936.
27. Cameron J., Bell, Grant Gall, D., Wallace, J.L. (1995) Disruption of colonic electrolyte transport in experimental colitis. Am. J. Physiol. 268: G622-G630.
28. Boni, B. (2002) Interneuron acquires worldwide rights to dersalazine for treatment of inflammatory bowel disease. Current News 781: 402-3410.
29. MacDermott, P.R, M.D. (2000) Progress in understanding the mechanisms of action of 5-amino salicylic acid. The Am. J. Gastroenterol. 95: 3343-3345.
30. MacDermott, P.R., Schloemann, S.R., Bertovich, M.J., . (1989) Inhibition of antibody secretion byb 5-aminosalicylic acid. Gastroentrology 96: 442-448.
31. Collier, H.O., Francis, A.A., McDonald-Gibson, W.J.,. (1976) Inhibition of prostaglandin biosynthesis by sulfasalazine and its metabolites. Prostaglandins 11: 217-224.
32. Sharon, P., Ligumsky, M., Rachmilewitz, D.,. (1978) Role of prostaglandins in ulcerative colitis. Enhanced production during active disease and inhibition by sulfasalazine. Gastroentrology 75: 638-640.