

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
21252

PHARMACOLOGY REVIEW

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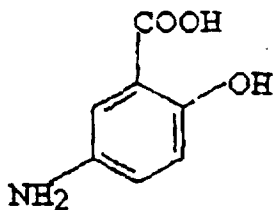
Date of HFD-180 Receipt: July 06, 2000

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REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA.

Drug: FIV-ASA (mesalamine) Suppository (500 mg).

The active ingredient of FIV-ASA is 5-aminosalicylic acid (5-ASA) also known as mesalamine. The chemical name of mesalamine is 5-amino-2-hydroxybenzoic acid.



C₇H₇NO₃

MW: 153.14

Category: Anti-inflammatory agent.

Proposed Marketing Indication: For the treatment of active ulcerative proctitis.

Dose: The dose of FIV-ASA Suppositories is 500 mg 3 times a day.

SUBMISSION CONTENTS:

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TOXICOLOGY Fourteen-Day intra-rectal toxicity study in rabbits.	2000-0074	9J44, 9K67, 0A016		8
Fourteen-Day intra-rectal toxicity study in Beagle dogs.	2000-0062	9J44, 9K67, 0A016		11
GENOTOXICITY In vitro mouse lymphoma forward gene mutation assay.	.000128	990356		15

The sponsor submitted NDA 21-252 for FIV-ASA (mesalamine suppositories for the treatment of ulcerative proctitis). The application was filed in accordance with Section 505 (b)(2). The sponsor conducted a 14-day intra-rectal toxicity study in rabbits and a 14-day intra-rectal toxicity study in beagle dogs with mesalamine suppositories. An *in vitro* mouse lymphoma forward gene mutation assay with mesalamine was also conducted. In their submission, the sponsor included all published preclinical studies on mesalamine up to April 2000. The submitted studies and the relevant published literature on Mesalamine were reviewed. In addition, the reviews of the pharmacology, absorption, distribution and metabolism (ADME) and toxicology studies submitted as part of NDA 19-618 (Rowasa enema, Reid-Powell Inc., Baudette, MN) and NDA 19-919 (Rowasa Suppositories, Reid-Rowell Inc., Marietta, GA), were included in the submission, and were consulted.

The following studies were submitted as part of NDA 19-618: Pharmacology, single dose toxicity studies, ocular irritation test, rectal mucosal irritation studies in rabbits and dogs, delayed contact hypersensitivity test, nephrotoxicity studies, multiple dose toxicity studies-13-week oral toxicity study in rats, 130 week oral (63-Week interim report) toxicity study in rats, 24-week oral toxicity study in dogs, reproduction studies- fertility and reproductive performance in rats, teratology studies in rats and rabbits, peri- and post-natal development in rats, mutagenicity studies- Ames tests, *in vivo* mouse bone marrow micronucleus assay and sister chromatid exchange assay in Chinese hamster bone marrow.

The following studies were submitted as part of NDA 19-919: a 127-week carcinogenicity study in rats and a 12-month eye toxicity study in dogs (Human Toxicol., 6: 377-383, 1987).

PHARMACOLOGY:

The sponsor submitted the published preclinical studies from 1986 to 2000 on the *in vitro* and *in vivo* pharmacological properties of mesalamine. The relevant studies are reviewed.

Antiinflammatory Effects of Various Drugs on Acetic Acid Induced Colitis in the Rat (Fitzpatrick et al., Agents and Actions 1990; 30: 393-402)

The effects of sulfasalazine, 5-ASA and other antiinflammatory agents on acetic acid induced colitis were studied in male Sprague-Dawley rats. Colitis was induced by intraluminal application of 5% acetic acid into the colon. Colonic mucosal myeloperoxidase activity was measured in mucosal scrapings by measuring the changes in the absorbance.

Oral administration of sulfasalazine (400 mg/kg) prior to induction of colitis caused significant reduction of the inflammatory response and mucosal myeloperoxidase activity (ED_{50} >400 mg/kg by the oral route). Pretreatment of the animals with 5-ASA (100 mg/kg) by the intra-rectal route also caused significant attenuation of the colonic myeloperoxidase activity (ED_{50} 71 mg/kg by the intrarectal route) and reduction of the lesion score. Thus, Sulfasalazine and its therapeutically active metabolite 5-ASA were effective in reducing acetic acid-induced colonic inflammation and colonic myeloperoxidase activity in rats.

Pharmacological Studies of BX661A, 5-[4-(2-Carboxyethylcarbamoyl)-phenylazo]-Salicylic Acid Disodium Salt Dihydrate (Kimura et al., Folia Pharmacol Jpa 1997; 109: 85-94)

The effects of intrarectal doses of 5-aminosalicylic acid (5-ASA), 5-aminobenzoyl- β -alanine (4-ABA) and sulfapyridine on dextran sulfate sodium (DSS)-induced ulcerative colitis in rats were studied. DSS (3%) was administered in drinking water for at least 10 days.

In the control animals, the length of the large intestine was shortened significantly and treatment of the animals with 5-ASA (35.0 and 105 mg/kg) caused significant improvement of the "large bowel shortening" (48% and 50% respectively). Treatment with 5-ASA also caused significant improvement of the erosion areas in the colon of DSS-treated animals as assessed by macroscopic examination of the colonic specimens. The 105 mg/kg was the most effective dose in the rat model. Thus, intrarectal 5-ASA was very effective reducing inflammation in DSS-induced colitis in rats.

The Anti-Oxidant Properties of 5-Aminosalicylic Acid (Pearson et al., Free Radical Biology & Medicine 1996; 21: 367-373)

The anti-oxidant properties of 5-ASA were studied in the guinea pig intestinal microvillus brush border membrane preparation. Parianaric acid was used as a fluorescent marker for oxidation. Oxidation was initiated from within the membrane by 2,2' azobis (2,4-dimethylvaleronitrile) (AMVN) and from solution by 2,2' azobis (2-amidinopropane) hydrochloride (AAPH). The anti-oxidant properties of 5-ASA were compared with that of tocopherols and ascorbate.

AMVN-induced lipid peroxidation within the microvillus membrane was dose dependently inhibited by 5-ASA (68.8% inhibition at 30 μ M). The concentration for half-maximal inhibition (ED_{50}) of lipid peroxidation was 1.5 μ M. Tocopherol was less effective (ED_{50} 12.4 μ M) while ascorbate had no effect on lipid peroxidation. Five-ASA was also very effective in inhibiting lipid peroxidation on the outer surface of the membrane vesicles. There was maximum inhibition of 90% with an ED_{50} of 4.4 μ M. Thus,

5-ASA caused inhibition of oxidation of the lipid membranes from both outside and within the cell membrane and this may be an important mechanism of cellular protection by oxidative stress.

Actions of Sulfasalazine and 5-Aminosalicylic acid as Reactive Oxygen Scavengers in the Suppression of Bile Acid-induced Increases in Colonic Epithelial Cell Loss and Proliferative Activity (Craven et al., Gastroenterology 1987; 92: 1998-2008)

The mechanism of suppression of mucosal injury by sulfasalazine and its therapeutically active metabolite 5-aminosalicylic acid (5-ASA) was examined in a rat model in which colonic epithelial cell loss and subsequent increases in epithelial proliferative activity was induced by intracolonic administration of sodium deoxycholate (DOC). The loss of DNA into the colonic lumen was used as a measure of colonic epithelial cell loss induced by DOC. Superoxide production in the colonic epithelium was measured by the cytochrome c reduction assay and ornithine decarboxylase activity of the colonic mucosal scrapings was measured from the release of $^{14}\text{CO}_2$ from radiolabeled ornithine. The effects of sulfasalazine (25 mM) and 5-ASA (25 mM) on DOC-induced red cell lysis were measured by absorbance spectroscopy.

Intraluminal instillation of DOC (5 mM) caused a six-fold increase in luminal DNA content within 60 minutes as compared with that in animals receiving saline. Sulfasalazine and 5-ASA completely prevented the DOC-induced increases in luminal DNA. In contrast, sulfapyridine had no effect on the DOC-induced increase in the luminal DNA content. Sulfasalazine and 5-ASA also blocked xanthine-xanthine oxidase-induced loss of DNA and subsequent proliferative response in the colonic epithelium. There were significant increases in ^3H -Thymidine incorporation into rat colonic luminal DNA after treatment with DOC for 12 hours, and treatment with 5-ASA and sulfasalazine blocked this effect of the bile acid. The increases in the superoxide anion (O_2^-) production by the bile acid were also abolished by 5-ASA (25 μM) and sulfasalazine (500 μM). These protective effects of 5-ASA and sulfasalazine may play a role in their therapeutic effects of inflammatory bowel disease.

Actions of Phenolic Derivatives (Acetaminophen, Salicylate, and 5-Aminosalicylate) as Inhibitors of Membrane Lipid Peroxidation and as Peroxyl Radical Scavengers (Dinis et al., Arch Biochem Biophys 1994; 315: 161-169)

The effects of acetaminophen, salicylate and 5-aminosalicylate (5-ASA) on lipid peroxidation were examined *in vitro*. The anti-oxidant properties of these compounds was determined with diphenylpicrylhydrazyl (DPPH) from the change in absorbance at 517 nm. Lipid peroxidation in rabbit muscle sarcoplasmic reticulum (SR) membranes by Fe^{2+} /ascorbate was measured by gas-liquid chromatography.

Among the three compounds 5-ASA was the most effective in causing reduction of DPPH. Iron/ascorbate-induced lipid peroxidation in SR was concentration dependently inhibited by 5-ASA. The peroxidative degradation of the lipid membranes was significantly higher with 5-ASA as compared with acetaminophen and salicylate. Five-ASA was also found to be a strong peroxyl radical scavenger in rabbit SR membranes (about 80% decreases at 0.5 mM 5-ASA). The data suggest that one of the mechanisms of

the anti-inflammatory actions of 5-ASA in inflammatory bowel disease may be by protecting the colonic mucosa from the free-radical induced damage.

5-Aminosalicylate: Oxidation by Activated Leukocytes and Protection of Cultured Cells from Oxidative Damage (Dull et al., Biochem Pharmacol 1987; 36: 2467-2472)

The mechanisms of action of 5-ASA in inflammatory bowel disease was studied by its ability to bind reactive hydroxyl radicals and to protect cultured Chinese hamster (CHO) cells from the lethal effects of superoxide radical or hydrogen peroxide.

Activated mononuclear cells and activated granulocytes, as well as hydroxyl radical generated by Fenton reaction oxidized ^{14}C -5-ASA to a number of metabolites. Five-ASA (0.65 mM) protected the cultured CHO cells from the lethal effects of superoxide radical or hydrogen peroxide. Superoxide dismutase had similar protective effects on the CHO cells. Thus, 5-ASA was oxidized by the products of oxidative burst of white blood cells, and 5-ASA protected the CHO cells from damage caused by free radicals, which may explain the mechanism of action of 5-ASA in inflammatory bowel disease.

Primary Colonic Epithelial Cell Culture of the Rabbit Producing Prostaglandins (Hata et al., Prostaglandins 1993; 45: 129-141)

The effects of 5-ASA and indomethacin on prostaglandin production were examined in cultured colonic epithelial cells from Japanese white rabbits. Collagenase digested colonic epithelial cells were grown in Modified Ham's F-12 medium and prostaglandin E_2 (PGE_2) and prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$) production by the cultured cells were quantified by radioimmunoassay before and after treatment with 5-ASA (200 μM to 5 mM) or indomethacin (10 nM to 1 μM).

Rabbit colonic epithelial cells in primary culture produced prostaglandin E_2 and treatment of the cells with 5-ASA for 30 minutes caused dose-dependent reduction of the prostaglandin production. Indomethacin had similar inhibitory effect on PGE_2 production in cultured epithelial cells. The findings suggest that 5-ASA may exert its antiinflammatory action by inhibiting the production of inflammatory prostaglandins.

Participation of Thromboxane and Other Eicosanoid Synthesis in the Course of Experimental Inflammatory Colitis (Vilaseca et al., Gastroenterology 1990; 98: 269-277)

The role of the inflammatory eicosanoids on trinitrobenzene sulfonic acid (TNBS)-induced colonic inflammation and the effects of prednisone and 5-ASA on the inflammation and eicosanoid levels were examined in male Sprague-Dawley rats. Luminal eicosanoid release was quantified *in vivo* using a dialysis bag placed into the distal colon.

Three days after intracolonic injection of different doses of TNBS, there were significant increases of prostaglandin E_2 , 6-keto-prostaglandin $\text{F}_{1\alpha}$, thromboxane B_2 (TXB_2) and leukotriene B_4 release as compared with the controls. The increase in prostaglandin E_2 and leukotriene B_4 levels were significantly reduced (51.2% and 86.5% decreases respectively on Day 14) by 5-ASA (10% as enema).

The release of TXB₂ continued to increase during the chronic stage of inflammation (up to Day 21), while the levels of other eicosanoids declined. Treatment with 5-ASA reduced TXB₂ levels during the chronic stage of inflammation ($p < 0.01$). Five-ASA also improved the TNBS-induced morphological damage as assessed by macroscopically and histologically. Thus, in the rat model of chronic colonic inflammation, the anti-inflammatory effects of 5-ASA may be due to inhibition of eicosanoid release into the colonic mucosa.

Effect of Aminophenols (5-ASA and 4-ASA) on Colonic Interleukin-1 Generation (Rachmilewitz et al., Gut 1992; 33: 929-932)

The effects of 5-ASA and 4-ASA on trinitrobenzene sulfonic acid-induced colitis in rats and interleukin-1 (IL-1) generation in cultured biopsy samples from active ulcerative colitis patients were studied. The rats were treated with oral doses of 5-ASA (50 mg/kg) and 4-ASA (50 mg/kg) daily for one to three weeks after induction of colitis. The thromboxane B₂ (TxB₂), leukotriene B₄ (LTB₄), myeloperoxidase activity and IL-1 contents of the mucosal scrapings were determined by suitable methods.

Treatment with 5-ASA (50 mg/kg, for 3 and 1 weeks) and 4-ASA (50 mg/kg, for 3 weeks) caused significant reduction of colonic IL-1 generation (65.4% decrease) and the extent and severity of inflammation in the rat model of colitis. Five-ASA (25-100 µg/ml) also caused significant decrease in the tissue IL-1 content (62% of the control at 100 µg/ml) and its release into the culture medium from the cultured biopsy specimens (48% of the control at 100 µg/ml). The inhibition of IL-1 generation by 5-ASA has been suggested as one of its mechanisms of action in inflammatory bowel disease.

Effect of Mesalamine on the hsp 72 Stress Response in Rat IEC-18 Intestinal Epithelial Cells (Burruss et al., Gastroenterology, 1997; 113: 1474-1479)

The effects of mesalamine on the hsp 72 stress response was examined in IEC-18 intestinal epithelial cells after treating the cultured cells with mesalamine (0.3, 1 or 3 mmol/L). The expression of hsp 72 in response to heat shock were examined in the presence and absence of mesalamine at both mRNA and protein levels. The heat shock protein expression was quantified by Western blot and the mRNA was quantified by Northern blot analyses. Protection of the cells from oxidant injury was assessed by quantifying the release of ⁵¹Cr from ⁵¹Cr-labeled cells in response to treatment with NH₂Cl (0.3 mmol/L for 30 minutes).

Treatment of the IEC-18 intestinal cells with mesalamine (1 mmol/L) for 60 and 120 minutes caused a significant increase in the expression of hsp 72 stress protein in response to heat shock. The heat shock protein expression in mesalamine treated cells were $145 \pm 12\%$ and $176 \pm 21\%$ at 60 and 120 minutes respectively as compared with untreated controls. Mesalamine also caused a dose dependent increase in the expression of hsp 72 mRNA in response to heat shock ($151 \pm 13\%$ and $197 \pm 16\%$ of control at 1 and 5 mmol/L respectively). Thus, treatment with mesalamine caused augmentation of the thermal induction of the intestinal epithelial hsp 72 expression and this effect was accompanied by increased cellular protection against oxidant injury.

Mesalamine Blocks Tumor Necrosis Factor Growth Inhibition and Nuclear Factor κ B Activation in Mouse Colonocytes (Kaiser et al., Gastroenterology 1999; 116: 602-609)

The effect of mesalamine on tumor necrosis factor- α (TNF- α)-initiated signaling events was studied in adult mouse colonic cells. Colonic cells were cultured with murine TNF- α , EGF or additives for various periods of time. TNF- α was added to the medium 30 minutes after addition of mesalamine (200 mmol/L) and EGF was added 30 minutes after TNF- α . Tumor necrosis factor- α signaling pathway was studied by measuring the signal peptides such as mitogen activated protein kinase (MAP kinase) and I κ B α in the cell lysates by Western blot analysis.

Mesalamine (20 mmol/L) blocked the proliferation of mouse colonic epithelial cells caused by the high dose of TNF- α (100 ng/ml) as well as epidermal growth factor (EGF)-mediated mitogenesis by TNF- α . Treatment of the cells with mesalamine completely blocked the TNF- α -induced activation of MAP kinase (ERK1/ERK2). TNF- α -induced activation of NF- κ B by I κ -B α degradation and its nuclear translocation in mouse colonic epithelial cells were also blocked by mesalamine (20 mmol/L). Thus, mesalamine may exert its antiinflammatory action in chronic inflammatory state by inhibiting the critical signal transduction pathways by TNF- α .

In summary, 5-ASA reduced the inflammatory changes in experimental ulcerative colitis in different animal models, when administered by the oral or intra-rectal route. Although the mechanism of the anti-inflammatory action of 5-ASA is not clearly understood, studies by different authors suggest that the drug has modulatory effects on different inflammatory mediators, such as prostaglandins, interleukin-1 or thromboxane synthesis. It has been found to act as a reactive oxygen scavenger in bile acid induced colitis in rats and inhibit the tumor necrosis factor- α -induced growth and critical signal transduction pathways in the mouse colonocytes.

ABSORPTION, DISTRIBUTION AND METABOLISM:

Intestinal metabolism and transport of 5-aminosalicylate (5-ASA) in anesthetized rats was studied by Zhou et al (Drug Metabolism and Disposition 1999; 27: 479-485). Isotonic solutions of different concentrations of 5-ASA were perfused into the jejunum or ileum of the anesthetized animals. 14 C-labeled polyethylene glycol was added to the 5-ASA perfusates as a tracer and samples were collected every 15 minutes. The transport of 5-ASA across basolateral (BL) or apical (AP) membranes was measured in cultured Caco-2 cell monolayers. The quantitation of 5-ASA and its metabolite N-acetyl-5-ASA in intestinal tissue, plasma and intestinal perfusate was carried out by HPLC with fluorescent detection. Jejunal permeability of 5-ASA (7.5 mg/ml) was higher than its ileal permeability ($6.17 \pm 1.82 \times 10^{-6}$ and $0.04 \pm 1.36 \times 10^{-6}$ cm/sec respectively). N-acetyl-5-ASA levels were also higher (approximately 6 times higher) in the jejunal perfusate as compared with that in the ileal perfusate. At low concentration (0.075 mg/ml), the absorption of the drug from the jejunum was significantly reduced due to its increased metabolism, while at a high concentration (7.5 mg/ml), there was no significant impact on absorption. Tissue metabolite levels were higher than plasma metabolite levels at low 5-ASA concentrations while the reverse was the case at high drug concentrations. At low concentrations, the transport of 5-ASA from BL

to AP layers of Caco-2 cells was higher, whereas at high concentrations, the flux was independent of the directions.

Grisham and Granger (Dig Dis Sci 1989; 34: 573-578) measured the 5-aminosalicylic acid concentrations in the mucosal interstitium of the small and large intestine of the cat during luminal perfusion of the drug. The concentrations of 5-ASA and its metabolite N-acetyl-5-ASA in intestinal lymph, venous plasma and systemic arterial plasma were measured. The concentrations of 5-ASA in the intestinal venous samples (143 ± 30 $\mu\text{g/ml}$) were always higher than the intestinal lymph (43 ± 17 $\mu\text{g/ml}$) or systemic arterial samples (40 ± 11 $\mu\text{g/ml}$). In the colon, the venous plasma concentration was about 10 times higher than that of systemic arterial plasma concentrations. In the terminal ileum, 58% of the luminal 5-ASA was absorbed, while in the colon, only 3% of the drug/min was absorbed. Thus, in the cat, the rate of 5-ASA absorption from the terminal ileum was approximately 7 times higher than that in the colon. The concentration of N-acetyl-5-ASA in the lymph or plasma was always lower than 5% of the 5-ASA concentrations, suggesting low acetylation of the drug in cats.

Tjornelund et al (Xenobiotica 1991; 21: 605-612) studied the metabolism of 5-ASA in pigs and humans receiving intravenous 5-ASA (250 mg i.v.). In addition to the metabolite, N-acetyl-5-ASA, a new metabolite N-formyl-5-ASA was identified in the urine of pigs and the plasma of healthy volunteers receiving 5-ASA. The formyl derivative of 5-ASA was also formed in rat liver homogenate when 5-ASA and N-formyl-L- kynurenine were added to the incubate.

TOXICOLOGY:

A 14-Day Toxicity Study with FIV-ASA Follows Intra-Rectal Administration in Rabbits. (#2000-0074)

Testing Laboratory:

Study Start and Completion Dates: February 22, 2000 and
June 15, 2000

GLP and OAU Compliance Statement: The sponsor included a statement of compliance with GLP regulation and quality assurance unit.

Animals: Male (2.1-2.6 kg; approx. 11 weeks old)
Female (2.0-2.6 kg; approx. 11 weeks old)
New Zealand Rabbits—

Drugs Batch No: Salofalk® FIV-ASA Suppository 250, 500 and 1000 mg/suppository; (Batch no. 9J44, 9K67 and 0A016 respectively); Salofalk® Placebo suppository (Batch no. 0B022)

Methods: Twenty-four (24) male and female rabbits were randomly assigned to 4 groups (3/sex/group). Groups 1, 2, 3 and 4 received twice daily, intra-rectal doses of 0, 250, 500 and 1000 mg FIV-ASA (mesalamine) suppositories respectively (0, 500, 1000 and 2000 mg/day; 0, 200, 400 and 800 mg/kg/day, considering 2.5 kg body weight) for 14 days. The sponsor stated that the doses were selected on the basis of the available data on mesalamine. The control animals received placebo suppositories identical in size to that of 500-mg suppository. On Day 7 in the morning, one animal (#2501A) was dosed with 500 mg instead of 250 mg. Beginning on Day 4, any animal that rejected the suppository within 30 minutes of dosing was administered a second suppository. The animals were observed for clinical signs and mortality once a day and a detailed clinical examination was performed once a week. Food consumption was recorded daily and the body weights were recorded once a week. Ophthalmologic examinations (indirect ophthalmoscopy and slit lamp) were conducted once prior to beginning of dosing and at the end of the dosing period. Hematology, clinical chemistry and urinalysis investigations were conducted once before the start of dosing and at the end of the dosing period. On Day 15, all animals were sacrificed and complete necropsies performed. The weights of the following organs were recorded. Adrenals, brain, heart, kidneys, liver, pituitary, prostate, spleen, thymus, thyroids (with parathyroids) and uterus.

The following organs were fixed in neutral buffered formalin for histopathological examinations. Adrenals, aorta (thoracic), brain, cecum, colon, duodenum, epididymides, esophagus, eyes, femur & marrow, gall bladder, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mandibular & mesenteric), mammary gland, optic nerves, ovaries, pancreas, pituitary, prostate, rectum, salivary gland (mandibular), sciatic nerve, seminal vesicles, skeletal muscle, skin & subcutis, spinal cord, spleen, sternum & marrow, stomach, testes, thymus, thyroids (with parathyroids), tongue, trachea, urinary bladder, uterus, vagina.

For toxicokinetic analysis, blood samples were collected on Day 1 following the first dose and Day 14 following the last dose. The plasma was analyzed for 5-aminosalicylic acid and its metabolite N-acetylaminosalicylic acid by

Results:

Clinical Signs: There were no treatment-related clinical signs in any groups. There were rejections of suppositories by both males and females; the males had more frequent rejections than the females (65 vs. 34 occasions). The number of rejections in different groups from Day 1 to Day 14 is summarized in the table below.

Frequency of suppository rejections by rabbits in the control and treatment groups

Day	Control		250 mg/suppository		500 mg/suppository		1000 mg/suppository	
	AM	PM	AM	PM	AM	PM	AM	PM
2	0	0	0	1	1	2	2	3
3	1	3	1	3	0	7	0	2
4	0	0	0	0	0	3	0	6
5	0	0	0	0	1	2	0	4
6	0	2	0	0	2	2	0	2
7	0	0	0	1	1	2	0	2
8	0	0	0	0	0	2	0	2
9	0	2	0	0	0	0	3	2
10	0	0	1	0	2	1	0	2

12	0	1	0	0	0	0	0	2
13	0	2	0	0	0	2	0	4
14	1	4	0	2	2	2	0	4
Total	2	14	2	7	9	25	5	35

Mortality: There were no deaths of animals in any groups.

Body Weights: The mean body weights of the control male and female animals before initiation of dosing were 2.1 ± 0.12 and 1.8 ± 0.12 kg and at the end of dosing were 2.5 ± 0.15 and 2.3 ± 0.0 kg respectively. No treatment-related changes in the body weights were observed in any groups.

Food consumption: The sponsor provided the food consumption values in terms of percent change from the control. No treatment-related changes in the food consumption were observed in any groups.

Ophthalmoscopy: No treatment-related ophthalmologic changes were observed in any groups.

Hematology: The hematological parameters of the control males and females are summarized in the table below.

WBC ($\times 10^9/L$)	RBC ($\times 10^{12}/L$)	Hemoglobin (g/L)	Hematocrit (L/L)	MCV (fL)	MCHC (g/L)	Platelets ($\times 10^9/L$)	Neutrophil ($\times 10^9/L$)	Lymphocyte ($\times 10^9/L$)	Monocyte ($\times 10^9/L$)	Eosinophil ($\times 10^9/L$)
Males										
7.4 ± 1.34	6.4 ± 0.45	136 ± 7.8	0.64 ± 0.044	100 ± 3.95	214 ± 1.5	510 ± 42.7	0.65 ± 0.017	5.77 ± 1.22	0.40 ± 0.101	0.33 ± 0.042
Females										
5.9 ± 1.83	6.4 ± 0.12	132 ± 8.9	0.62 ± 0.025	97.8 ± 4.54	211 ± 5.9	487 ± 24.1	0.78 ± 0.303	4.35 ± 1.90	0.32 ± 0.075	0.20 ± 0.19

No treatment-related changes in the hematology parameters were observed in any groups.

Clinical Chemistry: No treatment-related changes in the clinical chemistry parameters were observed in animals receiving intra-rectal 5-ASA. The low dose animals had a decrease (33%) in the mean cholesterol levels as compared with the controls. However, there were no dose-related changes.

Urinalysis: No treatment-related changes in the urinalysis parameters were observed.

Gross Pathology: There were no treatment-related gross pathological changes in any groups.

Organ Weights: The mean pituitary (absolute) weights of group 4 animals were lower (20%) than the controls. No other significant changes in the organ weights were observed in any groups.

Histopathology: Subacute and necrotizing inflammation of the stomach wall was observed in one male and one female each in the 250 mg and 500 mg suppository groups and in one male in the 1000 mg suppository group. No microscopic lesions were observed in the rectum, colon or cecum of any animals.

Toxicokinetics: During the 14-day intra-rectal administration of FIV-ASA suppositories to rabbits, the active drug, 5-aminosalicylic acid (5-ASA) was absorbed into the systemic circulation. The metabolite of 5-ASA, N-acetylaminosalicylic acid (N-acetyl-5-ASA) was also detected in the plasma of the animals. The concentrations of both 5-ASA and N-acetyl-5-ASA increased with dose in both males and females. With multiple dosing, there was an increase in the plasma concentrations of N-acetyl-5-ASA, but there

was no apparent change in the concentration of 5-ASA. The toxicokinetic parameters of 5-aminosalicylic acid and N-acetyl-5-ASA in the male and female rabbits are summarized in the tables below.

Toxicokinetic parameters of 5-aminosalicylic acid in rabbits during 14-day intra-rectal administration.

Day 1	Males			Females		
	250 mg	500 mg	1000 mg	250 mg	500 mg	1000 mg
C _{max} (µg/ml)	0.90±0.51	1.26±0.22	7.65±6.05	1.21±0.53	3.57±2.65	4.53±2.80
T _{max} (h)	0.91±0.54	0.25±0.0	0.67±0.17	0.25±0.0	0.33±0.08	0.58±0.22
AUC _{0-8h} (µg.h/ml)	0.91±0.39	1.39±0.80	8.44±6.62	1.49±1.70	3.21±2.34	5.59±5.87
Day 14						
	250 mg	500 mg	1000 mg	250 mg	500 mg	1000 mg
C _{max} (µg/ml)	0.74±0.20	1.65±1.06	8.07±9.14	1.24±1.00	2.24±1.78	3.20±2.51
T _{max} (h)	0.33±0.08	0.33±0.08	1.58±1.21	1.83±1.09	4.33±3.83	0.33±0.08
AUC _{0-8h} (µg.h/ml)	0.74±0.21	2.00±1.63	32.11±51.04	2.63±1.09	2.47±1.40	2.97±2.56

Toxicokinetic parameters of N-acetyl-5-ASA in rabbits during 14-day intra-rectal administration.

Day 1	Males			Females		
	250 mg	500 mg	1000 mg	250 mg	500 mg	1000 mg
C _{max} (µg/ml)	1.91±1.44	2.99±1.35	15.00±4.56	2.56±1.62	5.45±1.59	9.31±6.86
T _{max} (h)	0.33±0.08	0.58±0.22	0.67±0.17	0.42±0.08	1.67±1.17	0.67±0.17
AUC _{0-8h} (µg.h/ml)	2.18±2.50	4.12±2.66	21.64±11.03	4.05±4.56	11.63±7.73	13.53±12.32
Day 14						
	250 mg	500 mg	1000 mg	250 mg	500 mg	1000 mg
C _{max} (µg/ml)	3.15±0.43	6.95±3.71	22.92±22.36	5.42±1.77	8.09±1.23	9.53±4.10
T _{max} (h)	0.33±0.08	8.41±7.79	1.67±1.17	8.50±7.75	0.17±0.17	8.50±7.75
AUC _{0-8h} (µg.h/ml)	6.22±1.53	11.26±9.19	111.95±158.9	17.12±9.71	23.14±5.15	24.51±14.06

Mean ± S.D.; n=3

In summary, in the 14-day toxicity study with FIV-ASA suppositories in rabbits, the animals (3/sex/group) received intra-rectal doses of 0, 250, 500 and 1000 mg twice daily (0, 500, 1000 and 2000 mg/day; approximately 0, 200, 400 and 800 mg/kg/day). Subacute and necrotizing inflammation of the stomach wall was observed in 1 male (of 3) and 1 female (of 3) each in the 250 and 500 mg dose groups and 1 male (of 3) in the 1000 mg dose group. Thus, the target organ of toxicity was the stomach and the 'no effect dose' was not identified. Five-ASA and its metabolite N-acetyl-5ASA were absorbed in the systemic circulation after intrarectal administration of the drug.

A 14-Day Toxicity Study with FIV-ASA Follows Intra-Rectal Administration in Beagle Dogs. (#2000-0062)

Testing Laboratory:

Study Start and Completion Dates: February 07, 2000 and June 15, 2000

GLP and QAU Compliance Statement: The sponsor included a statement of compliance with GLP regulation and quality assurance unit.

Animals: Male (9.8-14.3 kg; approx. 6-8 months old)
Female (8.3-13.4 kg; approx. 6-8 months old)
Beagle dogs

Drugs Batch No: Salofalk® FIV-ASA Suppository 250, 500 and 1000 mg/suppository: (Batch no. 9J44, 9K67 and 0A016 respectively); Salofalk® Placebo suppository (Batch no. 0B022)

Methods: Twenty-four (24) male and female beagle dogs were randomly assigned to 4 groups (3/sex/group). Groups 1, 2, 3 and 4 received twice daily intra-rectal doses of 0, 250, 500 and 1000 mg FIV-ASA suppositories respectively (0, 500, 1000 and 2000 mg/day; 0, 41.6, 83.3 and 166.6 mg/kg/day, considering 12 kg body weight of the dogs). The suppositories were administered twice daily (morning and afternoon) for 14 consecutive days. The control animals received placebo suppositories identical in size to that of 500-mg suppository. The animals that rejected a suppository within 30 minutes post-dosing was administered a second suppository. The animals were observed for clinical signs and mortality once a day; in addition, the animals were monitored for approximately 1 hour after dosing. Food consumption was recorded daily and the body weights were recorded once a week. Ophthalmologic examinations (indirect ophthalmoscopy and slit lamp) were conducted once before the beginning of dosing and at the end of the dosing period. Hematology, clinical chemistry and urinalysis investigations were conducted once before the start of dosing and at the end of the dosing period. For toxicokinetic study, blood samples were collected after the first dosing (morning) on Days 1 and 14 (once before dosing and at 15min, 30min, 1h, 2h, 4h, 6h and 8h post dosing). Blood samples were also collected at 12h and 24h after the first dosing on Day 14. The plasma concentrations of 5-aminosalicylic acid and N-acetylaminosalicylic acid were determined by

On Day 15, all animals were sacrificed and necropsies performed. The weights of the following organs were recorded.

Adrenals, brain, heart, kidneys, liver, pituitary, prostate, spleen, thymus, thyroids (parathyroids) and uterus.

The following tissues and organs were fixed in neutral buffered formalin for histopathological examinations:

Adrenals, anal glands, aorta (thoracic), brain (cerebral, cortex, midbrain, cerebellum & medulla), cecum, colon, epididymides, esophagus, eyes, femur & marrow, gall bladder, heart, kidneys, liver, lungs (with bronchi), lymph nodes (mandibular & mesenteric), mammary gland, optic nerves, ovaries, pancreas, pituitary, prostate, rectum, salivary gland (mandibular), sciatic nerve, skeletal muscle, skin & subcutis (inguinal), small intestine (duodenum, ileum, jejunum), spinal cord (cervical), spleen, sternum & marrow, stomach, testes, thymus, thyroids (with parathyroids), tongue, trachea, urinary bladder, uterus, vagina.

Results:

Clinical Signs: One (of 3) male and 2 (of 3) female animals of the 1000 mg suppository group had vomiting during the dosing period (Day 3 to Day 15): One female animal of the 500 mg dose group had swelling of the anus on Day 15 and another female of the 250 mg dose group had loss of fur, a small scab and slight redness on the right axillary (Days 8-15). There were rejections of suppositories by both males and females; the males had more frequent rejections than the females (7 vs. 1 occasions). The number of rejections in different groups from Day 1 to Day 14 of dosing is summarized in the table below.

Frequency of suppository rejections by dogs in the control and treatment groups

Day (sex)	Control		250 mg/suppository		500 mg/suppository		1000 mg/suppository	
	AM	PM	AM	PM	AM	PM	AM	PM
1 (M)	0	0	0	1	0	0	0	0
7 (M)	0	0	0	0	1	0	0	0
9 (F)	1	0	0	0	0	0	0	0
13 (M)	0	0	1	0	2	0	0	1
14 (M)	0	0	1	0	0	0	0	0
Total	1	0	2	1	3	0	0	1

Mortality: There were no deaths of animals in any groups.

Body Weights: The mean body weights of the control male and female animals before initiation of dosing were 11.9 ± 0.46 and 11.6 ± 1.21 kg, and at the end of dosing were 12.6 ± 0.36 and 11.5 ± 1.47 kg respectively. No treatment-related changes in the body weights were observed in any groups.

Food consumption: The mean food consumption of the control male and female animals before the start of dosing were 400 ± 0.0 g and 378 ± 38.1 g/day respectively. No treatment related changes in the food consumption were observed in any groups.

Ophthalmoscopy: No treatment-related ophthalmologic changes were observed in any groups.

Hematology: The hematological parameters of the control male and female animals are summarized in the table below.

WBC ($\times 10^9/L$)	RBC ($\times 10^{12}/L$)	Hemoglobin (g/L)	Hematocrit (L/L)	MCV (fL)	MCHC (g/L)	Platelets ($\times 10^9/L$)	Neutrophil ($\times 10^9/L$)	Lymphocyte ($\times 10^9/L$)	Monocyte ($\times 10^9/L$)	Eosinophil ($\times 10^9/L$)
Males										
9.4 ± 2.50	6.8 ± 0.15	161 ± 1.5	0.45 ± 0.012	65.2 ± 0.82	362 ± 8.5	357 ± 47.7	5.84 ± 1.738	2.18 ± 0.723	1.04 ± 0.348	0.26 ± 0.114
Females										
9.8 ± 0.93	7.1 ± 0.30	169 ± 9.1	0.47 ± 0.020	65.8 ± 0.25	361 ± 4.0	347 ± 4.0	6.11 ± 1.363	2.70 ± 0.225	0.73 ± 0.544	0.19 ± 0.172

No treatment-related changes in the hematology parameters were observed in any groups.

Clinical Chemistry: No treatment-related changes in the clinical chemistry parameters were observed in animals receiving intra-rectal 5-ASA. A slight decrease in the mean glucose level (16.13%) was observed in the high dose females (5.2 mmol/L) as compared with the controls (6.2 mmol/L).

Urinalysis: No treatment-related changes in the urine parameters were observed.

Gross Pathology: There were no treatment-related gross pathological changes in any groups.

Organ Weights: The mean brain weights (relative) of the female animals of the 500 mg and 1000 mg suppository groups were significantly higher than controls (22.22% and 30.4% increases in the 500 mg and 1000 mg groups respectively). No other significant changes in the organ weights were observed in any groups.

Histopathology: Chronic nephritis and pyelitis was observed in one (of 3) female animal of the 1000-mg suppository group. Two females (of 3) of the same group had chronic inflammation of the lung and one female had moderate hyperplasia of the mammary gland. No microscopic changes were observed in the rectum, colon or cecum of any animals.

Toxicokinetics: Following intra-rectal administration of FIV-ASA suppositories to dogs, 5-ASA was detectable in the blood on Day 1 and Day 14 of dosing. The peak plasma concentration was reached within 6 hours of administration. The plasma levels increased with increasing doses in the males but not in the females. The maximum plasma concentrations on Day 14 were higher than that on Day 1 except in the high dose group. The $t_{1/2}$ values ranged from 1.8 to 3.8 hours in the males and 1.8 to 2.4 hours in the females. The metabolite of 5-ASA, N-acetylaminosalicylic acid was undetectable on Day 1 and was only detectable in some animals on Day 14. The toxicokinetic parameters of 5-aminosalicylic acid and N-acetylaminosalicylic acid in the male and female dogs are summarized in the tables below.

Toxicokinetic parameters of 5-aminosalicylic acid in dogs during 14-day intra-rectal administration.

Day 1	Males			Females		
	250 mg	500 mg	1000 mg	250 mg	500 mg	1000 mg
C_{max} ($\mu\text{g/ml}$)	1.943 \pm 1.136	2.920 \pm 0.481	5.373 \pm 4.375	2.041 \pm 1.331	4.415 \pm 0.502	3.430 \pm 1.38
T_{max} (h)	2.00 \pm 0.50	2.00 \pm 0.0	2.16 \pm 1.01	1.5 \pm 0.58	3.0 \pm 1.0	1.67 \pm 0.33
AUC_{0-8h} ($\mu\text{g}\cdot\text{h/ml}$)	8.355 \pm 1.896	22.138 \pm 3.78	14.63 \pm 2.157	8.885 \pm 5.680	23.915 \pm 6.54	26.157 \pm 17.2
Day 14						
	250 mg	500 mg	1000 mg	250 mg	500 mg	1000 mg
C_{max} ($\mu\text{g/ml}$)	2.377 \pm 0.38	8.437 \pm 6.470	3.973 \pm 3.344	3.170 \pm 0.963	6.880 \pm 4.971	27.92 \pm 40.51
T_{max} (h)	4.67 \pm 1.76	3.33 \pm 1.76	2.33 \pm 0.88	2.33 \pm 0.88	2.33 \pm 0.88	2.00 \pm 1.16
AUC_{0-8h} ($\mu\text{g}\cdot\text{h/ml}$)	13.705 \pm 2.28	40.90 \pm 21.09	20.874 \pm 18.92	15.842 \pm 4.286	29.969 \pm 5.18	124.98 \pm 173.44

Mean \pm S.D., n=3

In summary, in the 14-day toxicity study with FIV-ASA suppositories in beagle dogs, male and female animals (3/sex/group) received twice daily intra-rectal doses of 0, 250, 500 and 1000 mg (0, 500, 1000 and 2000 mg/day; approximately 0, 41.6, 83.3 and 166.6 mg/kg/day). Vomiting was observed in the high dose males and females during the entire dosing period. One (of 3) high dose female had chronic nephritis and pyelitis. Two females of the same group had chronic inflammation of the lung and another female had moderate hyperplasia of the mammary gland. The target organs of toxicity were the kidney and the lung and the 'no effect dose' was 1000 mg/day (83.2 mg/kg/day). After intra-rectal administration of 5-ASA suppositories to male and female dogs, the drug was absorbed systemically and the C_{max} and

AUC values achieved on Day 14 was higher than that on Day 1, which suggests that the steady state was not reached after the first dose.

GENOTOXICITY:

In Vitro Mouse Lymphoma Forward Gene Mutation (At The TK Locus) Assay With 5-ASA (Study Report # CIR000128)

Testing Laboratory:

Study Start and Completion Dates: April 14, 2000 and
May 25, 2000

GLP and OAU Compliance Statement: The sponsor included a statement of compliance with GLP regulation and quality assurance unit.

Cells Used: Mouse Lymphoma L5178Y cell line (American Type Culture Collection, lot # 206271), heterozygous at the tyrosine kinase (TK) locus was used for the assay.

Concentrations Used: The concentrations used, both with and without metabolic activation, were 0.1, 0.3, 1.0, 3.0, 10.0, 30.0, 100, 300 and 1000 µg/ml. The vehicle for the drug was 0.1N hydrochloric acid.

Basis of Dose Selection: The concentrations were selected on the basis of solubility of 5-ASA. The compound was found to be soluble at a concentration up to 100 mg/ml in 1N HCl (to be shaken for about 2 minutes at 37°C). After solubilization, working solutions at concentrations ranging 0.010 and 100 mg/ml were prepared, and a volume of 100 µl to each culture gave final concentrations ranging between 0.1 and 1000 µg/ml. These concentrations of 5-ASA were not cytotoxic to the L5178Y cells, as there was no decrease in the relative suspension growth during the 48-hour expression time.

Negative Control: As negative control, the solvent (0.1N HCl) alone both with and without metabolic activation was used.

Positive controls: Methylmethanesulfonate, at a final concentration of 10.0 µg/ml was used in the absence of metabolic activation, while cyclophosphamide, at a final concentration of 6.5 µg/ml was used in the presence of metabolic activation.

Metabolic Activation System: Aroclor 1254-induced rat liver S9 mix was used as the metabolic activation system.

Drugs Batch No: 5-Aminosalicylic acid (5-ASA); Lot no. 990356 (purity 99.0-101.0%).

Criteria for Considering the Test Positive: The test result was considered positive when one or more concentrations produced a doubling of the normal spontaneous mutant frequency at a survival of 10% or greater, with a dose-response relationship.

Methods: L5178Y cells were grown in culture at 37°C under 5% CO₂ in F_{10P} (Fisher's medium containing 0.2 mg/ml pyruvate, 1.8 mg/ml pluronic F-68 and 10% heat inactivated horse serum). The cells were divided at a concentration of 1x10⁶ cells/ml and treated with the test article and the negative and positive controls (with or without metabolic activation). After 4 hours treatment, the cells were washed and resuspended in the fresh medium. The cells were then incubated at 37°C under 5% CO₂ for an expression time of 61±3 hours and the cells were cloned to quantify the number of viable cells and the mutant frequency for each cultures. The mutants were scored as colonies arising from cells placed in soft agar containing 100 µg/ml of trifluorothymidine (TFT) and the viable cells were scored from colonies arising from medium without TFT. Mutant frequencies were expressed as the number of mutant cells per number of surviving cells.

Results: No toxicity of the test substance was observed up to the highest concentration (1000 µg/ml) tested as there was no decrease in the relative suspension growth with increasing concentrations. Both in the main mutagenicity assay and the repeat assay, the spontaneous mutant frequencies (F(x10⁶)) of the negative controls were lower than 100 TFT-resistant mutants per 10⁶ surviving cells. For the positive controls the spontaneous mutant frequencies were at least 100 per 10⁶ clonable cells above that of the negative controls. In the main mutagenicity assay, the mutant frequencies of the cultures treated with 1000 µg/ml of 5-ASA (in the presence of metabolic activation) and 10 µg/ml (in the absence of metabolic activation) were higher than 2-fold of the control mutant frequency. However, there was no dose-response relationship. In the repeat mutagenicity assay at these concentrations, the mutant frequency was lower than 2-fold of the spontaneous mutant frequency. The relative viable counts, relative total growths, the TFT-resistant counts and the mutant frequencies in different treatment groups and the negative and positive controls for the main mutagenicity assay and the repeat test are summarized in the table below.

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Main Mutagenicity Assay							
Agents	Conc (µg/ml)	S9 Mix	Viable Count (mean±S.D)	Relative Total Growth (%)	TFT resistant colonies (mean)	Mutant Frequency (F(x10 ⁶))	F (Induced)
Vehicle control (0.1 N HCl)	-	+	89.0±6.2	110.3	21.7	51	0
Vehicle control (0.1N HCl)	-	+	74.0±7.0	90.2	19.5	52	1
5-ASA	0.100	+	(1)	(1)	(1)	(1)	(1)
5-ASA	0.300	+	67.0±7.5	83.6	18.3	55	4
5-ASA	1.00	+	60.7±5.8	65.5	8.7	29	-22
5-ASA	3.00	+	52.7±3.2	43.8	12.0	46	-5
5-ASA	10.00	+	34.0±2.0	41.5	11.7	69	18
5-ASA	30.00	+	28.3±4.9	25.4	4.7	33	-18
5-ASA	100.0	+	33.0±6.1	28.5	12.0	73	22
5-ASA	300.0	+	33.3±1.5	39.3	7.3	44	-7
5-ASA	1000.0	+	28.0±6.2	35.7	16.0	114	63
Cyclophosphamide	5.60	+	20.3±2.3	23.9	35.7	351	318
Vehicle control (0.1N HCl)	-	-	99.7±7.4	92.1	34.0	68	2
Vehicle control (0.1N HCl)	-	-	105.3±21.7	108.2	34.0	65	-2
5-ASA	0.100	-	89.0±7.0	67.7	0.0	0	-66
5-ASA	0.300	-	108.0±2.6	93.9	30.0	56	-11
5-ASA	1.00	-	112.7±11.1	89.5	30.7	54	-12
5-ASA	3.00	-	111.3±8.6	85.9	31.0	56	-11
5-ASA	10.00	-	40.7±12.0	45.4	37.6	182 (3)	116
5-ASA	30.00	-	47.3±12.1	34.0	9.3	39	-27
5-ASA	100.0	-	0.0±0.0	(2)	7.7	(2)	(2)
5-ASA	300.0	-	0.0±0.0	(2)	(4)	(4)	(4)
5-ASA	1000.0	-	0.7±0.6	(2)	1.7	(2)	(2)
Methylmethanesulfonate	10.0	-	21.0±1.6	11.3	47.3	451	384

(1), the culture was not plated because of bacterial contamination; (2), was not calculated because the values were too low; (3), the culture was repeated; (4), the culture was not counted because of bacterial contamination.

Repeat Assay							
Agents	Conc (µg/ml)	S9 Mix	Viable Count (mean±S.D)	Relative Total Growth (%)	TFT resistant colonies (mean)	Mutant Frequency (F(x10 ⁶))	F (Induced)
Vehicle control (0.1N HCl)	-	+	101.7±4.0	105.1	25.3	50	6
Vehicle control (0.1N HCl)	-	+	83.0±6.0	93.9	15.3	37	-6
5-ASA	1000	+	94.3±7.0	72.1	39.3	83	40
Cyclophosphamide	6.5	-	(1)	(1)	(1)	(1)	(1)
Cyclophosphamide	6.5	+	70.7±8.4	33.5	58.3	165	122
Vehicle control (0.1N HCl)	-	-	62.0±3.6	80.1	14.3	46	3
Vehicle control (0.1N HCl)	-	-	50.3±4.7	114.2	10.3	41	-3
5-ASA	10	-	55.7±6.7	93.6	6.3	23	-21
5-ASA	30	-	102.7±9.2	196.1	27.7	54	10
5-ASA	100	-	66.7±7.5	116.5	16.0	48	4
5-ASA	300	-	108.7±6.1	157.0	18.3	34	-10
5-ASA	1000	-	62.3±6.7	100.0	13.0	42	-2
Methylmethanesulfonate	10.0	-	31.0±1.7	20.6	65.0	419	376
Methylmethanesulfonate	10.0	-	48.3±5.5	60.5	83.3	345	301

(1), the culture was not counted because of bacterial contamination.

Thus, the results of the assay indicates that 5-ASA was not mutagenic in the mouse lymphoma TK assay in the presence or absence of metabolic activation under the experimental conditions.

Proposed Text for the Labeling of Mesalamine Suppositories

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Proposed Labeling

SUMMARY AND EVALUATION:

The etiology of the ulcerative colitis is not clear. Sulfasalazine has been used in the treatment of ulcerative colitis for many years. In the colon, sulfasalazine is split by bacteria into sulfapyridine and mesalamine (5-ASA). It is believed that mesalamine is the only therapeutic component active in ulcerative colitis. Mesalamine is considered as an effective therapy for the treatment of ulcerative colitis. Although the mechanism of action of mesalamine is not clear, its action appears to be topical rather than systemic. So, the rectal administration of the drug is more efficient than oral dosing because of less systemic availability. The sponsor submitted NDA 21-252 for mesalamine suppositories (FIV-ASA Suppositories) for the treatment of ulcerative proctitis. The application was filed in accordance with Section 505 (b)(2).

In support of the application, the sponsor submitted full reports of (a) a 14-day intra-rectal toxicity study with FIV-ASA suppositories in rabbits, (b) a 14-day intra-rectal toxicity study with FIV-ASA suppositories in beagle dogs and (c) *in vitro* mouse lymphoma forward gene mutation assay with 5-ASA. In addition, summaries of the following studies submitted as part of NDA 19-618 for Rowasa enema and NDA 19-919 for Rowasa suppositories were submitted: Pharmacology, acute oral and IV toxicity study in mice and rats, 28-day palatability study in rats, 13-week oral toxicity study in rats, 6-month oral toxicity study in dogs, one year oral toxicity study in rats, 127-week carcinogenicity study in rats, mutagenicity studies-Ames test, mouse micronucleus test, sister chromosome exchange in Chinese hamster bone marrow cells, reproductive studies- oral segment I study in rats, oral segment II studies in rats and rabbits, oral segment III study in rats, special toxicity studies- eye irritation in rabbits, delayed contact hypersensitivity in guinea pigs, rectal tolerability in rabbits, rectal irritancy in dogs. The three full new studies submitted are reviewed; in addition, the pharmacology/toxicology reviews of NDA 19-919 (Rowasa Suppositories, Reid-Rowell Inc.) and NDA 19-618 (Rowasa enema), and the scientific literature published on mesalamine through April 2000 were consulted.

Although the mechanism of the anti-inflammatory action of 5-ASA is not clearly understood, studies by different authors suggest that the drug has modulatory effects on different inflammatory mediators, such as interleukin-1 production, thromboxane synthesis, nitric oxide synthase and prostaglandin production. Five-ASA has been found to cause a reduction of colonic interleukin-1 production in the rat model of colitis, reduction of *in vivo* thromboxane release during chronic phase of colonic inflammation and inhibition of eicosanoid synthesis in patients with IBD and inhibition of nitric oxide synthase in chronic colitis in animal models. Five-ASA has also been shown to inhibit PGE₂ production and protect cells from oxidant injury by acting as an intracellular/intranuclear antioxidant. It has been found to act as a reactive oxygen scavenger in bile acid induced colitis in rats and this has been postulated as a mechanism of anti-inflammatory action of the compound. Five-ASA has also been shown to inhibit the tumor necrosis factor- α -induced growth and signal transduction pathways in the mouse colonocytes.

After oral administration to rats, 5-ASA is rapidly absorbed in the proximal intestine and the parent compound and its metabolite, N-acetyl-5-ASA are mainly eliminated in the urine (>65%); only small proportions are excreted in the feces (<10%). The rate of absorption of the drug in the terminal ileum is approximately 7 times higher than that in the colon. Zhou et al. (Drug Metab Dispos, 1999, 27: 479-485), examined the characteristics of intestinal absorption and metabolism of 5-ASA in anesthetized rats and found that the drug is almost completely absorbed in the upper small intestine (jejunum) as compared to the ileum which is less permeable to the drug. The metabolite (N-acetyl-5-ASA) levels in the

intestinal tissue were higher than plasma levels at low drug concentrations, while the situation was reverse at higher drug concentrations. The authors found that at luminal levels of below 200 µg/ml, intestinal secretion of 5-ASA accounts for more than 50% of the total elimination and can significantly affect tissue levels. After intra-rectal administration in rabbits and dogs, 5-ASA and its metabolite N-acetyl-5-ASA was absorbed in systemic circulation and was detected in the plasma. In the rabbit, the plasma concentrations of the parent drug and the metabolite increased with increasing doses, but in the dogs, this was not always the case. The drug is largely eliminated as its acetylated metabolite N-acetyl-5-ASA that is predominantly excreted in the urine. In addition to the acetylated metabolite, a new metabolite of 5-ASA (N-formyl-5-ASA) was identified in the urine of pigs after oral administration and in the plasma of humans after IV administration (Tjornelund et al., *Xenobiotica*, 1991, 5: 605-612).

In the acute oral toxicity studies with 5-ASA, the highest non-lethal dose was 900 mg/kg in rats and less than 800 mg/kg in mice. The LD₅₀ values in rats and mice after oral dosing were 4196 mg/kg and 1104-1114 mg/kg respectively. Deaths occurred within 6-7 days after oral dosing and the signs of acute toxicity included sedation, dyspnea, ventral body position, weight loss and ruffled fur in rats and sedation, dyspnea, ventral position and tremor in mice. In acute IV toxicity study in rats, only one dose (2000 mg/kg) was tested; so the LD₅₀ was not identified. In mice, the highest non-lethal IV dose was less than 1000 mg/kg and the LD₅₀ was greater than 3000 mg/kg. Mortality occurred within 2 days after IV administration and the clinical signs were similar to those seen in oral toxicity studies. Macroscopic examinations revealed the kidney, GI tract and lung to be the target organs of toxicity.

In the 28-day palatability study in rats, the animals received 0, 100, 300 and 900 mg/kg/day doses, given in the diet. There were no treatment-related signs of toxicity or gross pathological findings in any groups.

In a renal toxicity study with 5-ASA in female rats, a single intravenous injection of 214.4, 428.8 or 872 mg/kg to the animals was associated with necrosis of the proximal convoluted tubules and the renal papilla at all dose levels (*Br. Med. J.* 1:152-154, 1972).

In the 13-week oral toxicity study with 5-ASA in rats, groups of animals received oral doses of 0, 40, 160 and 640 mg/kg/day. There were deaths at the 640 mg/kg/day dose. There was a marked accumulation of 5-ASA and its metabolite acetyl-5-ASA following 13-week repeated administration in rats. Renal toxicity was observed in animals in all treatment groups. Focal hemorrhagic erosions of the gastric mucosa, myocardial necrosis and erythropoietic depression in the bone marrow were observed in the 640 mg/kg/day group. The target organs of toxicity were the kidney, GI tract, heart and the hematopoietic system and the 'no effect dose' was not established.

In the 24-week oral toxicity study in dogs, the animals received oral doses of 0, 40, 80 and 120 mg/kg/day of the drug. There were no deaths in any groups. The target organ of toxicity was the kidney and the 'no effect dose' was 40 mg/kg/day.

In the one-year oral toxicity study in rats, male and female animals received daily 5-ASA doses of 0, 50, 100 and 320 mg/kg/day. There were no deaths in any groups. Suppression of body weight gains was observed in the females receiving the 320 mg/kg/day dose. Degeneration of the kidney was observed in the 100 and 320 mg/kg/day groups. The target organ of toxicity was the kidney and the 'no effect dose' was 50 mg/kg/day.

In the 127-week oral carcinogenicity study in rats, male and female animals received 0, 50, 100 and 320 mg/kg/day doses of 5-ASA. Doses were selected on the basis of a 13-week oral toxicity study. Although, mortality was high at the end of the study period, it was less than 50% at week-104. The target organs of toxicity were the kidney and the GI tract. The dose selection for the study was appropriate. The study is valid and acceptable. There was no indication of tumorigenic potential for 5-ASA in male and female rats.

In a study by Hirouchi et al (J Toxicol Sci., 23: 539-552, 1998), the liver tumor promoting effects of 5-ASA was assessed in rats treated with diethylnitrosamine (DEN). Five-ASA was administered at oral doses of 150 and 300 mg/kg/day for 6 weeks. Five-ASA was not found to have any tumor promoting effect on this animal model, although the positive control, phenobarbital, caused a significant increase in the DEN- induced tumorigenesis.

In the oral segment I fertility and reproductive performance study in the male and female rats, 5-ASA was administered at oral doses of 0, 80, 160 and 320 mg/kg/day. Five-ASA, at oral doses up to 320 mg/kg/day (about 1.7 times the recommended human intra-rectal dose, based on body surface area) did not affect fertility or reproductive performance in the rat. However, the sponsor did not perform any interim sacrifice of the dams on Day-13 of pregnancy to detect any effects on the early events of pregnancy.

In the oral segment II teratology studies with 5-ASA, the drug was administered at 0, 80, 160 and 320 mg/kg/day doses (during days 6-15 of the gestation period) to rats and at 0, 55, 165 and 495 mg/kg/day doses to rabbits (during days 6-18 of the gestation period). Five-ASA, at oral doses up to 320 mg/kg/day (about 1.7 times the recommended human intra-rectal dose, based on body surface area) in rats and up to 495 mg/kg/day (about 5.4 times the recommended human intra-rectal dose, based on body surface area) in rabbits had no parental, embryotoxic or teratogenic effects.

In the segment III peri- and postnatal development study in rats, 5-ASA was administered at oral doses of 0, 80, 160 and 320 mg/kg/day from Day-16 of gestation through lactation. The compound, at doses up to 320 mg/kg/day (about 1.7 times the recommended human intra-rectal dose, based on body surface area), had no influence on any reproductive parameters and was devoid of any maternal or fetal toxicities.

The genotoxic potential of 5-ASA was examined in the Ames tests, the *in vitro* mouse lymphoma cell (TK⁺) forward gene mutation assay and the *in vivo* mouse micronucleus assay. Five-ASA was not mutagenic in the above mentioned *in vitro* and *in vivo* mutagenicity assays.

The mutagenic potential of 5-ASA was also examined by Witt et al (Mutation Research, 283: 59-64, 1992) in *in vitro* sister chromatid exchanges (28-280 µg/ml) and chromosomal aberrations in Chinese hamster ovary (CHO) cells (61-280 µg/ml), and in *in vivo* mouse bone marrow micronucleus assay (125-250 mg/kg). Five-ASA was found to be negative in all three *in vitro* and *in vivo* genotoxicity assays.

In special toxicity studies, 5-ASA was found to cause only mild to slight mucosal irritation of the eyes of New Zealand rabbits, when 0.1 g of the drug was applied to the conjunctival sac. No drug-induced discoloration of the cornea and conjunctiva or corrosion of the cornea was observed. Five-ASA

was non-sensitizing in the guinea pig delayed hypersensitivity test. In the suppository dosage form, it was tolerated by the rectal mucosa of New Zealand rabbits following daily intra-rectal administration for 5 days (0.25 g/suppository). Five-ASA rectal suspension (~2.1 g 5-ASA) was well tolerated by the rectal mucosa of mongrel dogs on repeated administration up to 28 days.

In the 14-day toxicity study with 5-ASA suppositories in rabbits (Axcen Scandipharm Inc.), male and female animals (3/sex/group) received twice daily intra-rectal doses of 0, 250, 500 and 1000 mg (0, 500, 1000 and 2000 mg/day; approximately 0, 200, 400 and 800 mg/kg/day) 5-ASA. Subacute and necrotizing inflammation of the stomach wall was observed in 1 male (of 3) and 1 female (of 3) each in the 250 and 500 mg suppository groups and 1 male (of 3) in the 1000 mg dose group. The target organ of toxicity was the stomach and the 'no effect dose' was not established.

In the 14-day toxicity study with 5-ASA suppositories in dogs (Axcen Scandipharm Inc.), the animals (3/sex/group) received twice daily intra-rectal doses of 0, 250, 500 and 1000 mg (0, 500, 1000 and 2000 mg/day; approximately 0, 41.6, 83.3 and 166.6 mg/kg/day) of 5-ASA. Vomiting was observed in the high dose males and females during the dosing period. One (of 3) high dose female had chronic nephritis and pyelitis. Two females of the same group had chronic inflammation of the lung and another female had moderate hyperplasia of the mammary gland. The target organs of toxicity were the kidney and the lung and the 'no effect dose' was 1000 mg/day (83.3 mg/kg/day).

In the 12-month eye toxicity study with 5-ASA in dogs, groups of male and female animals (6/sex/group) received 0, 40, 60 and 100 mg/kg/day oral doses of the drug. Keratoconjunctivitis sicca (an inflammatory eye condition involving mucopurulent discharge and corneal ulcerations) was observed at 40 mg/kg/day and higher doses; the condition was observed as early as week-9 of the dosing period. The females were affected earlier and more severely than the males. Keratoconjunctivitis sicca in dogs has also been reported in a published study where the animals received oral doses of 5-ASA for 12 months (Human Toxicol. 6: 377-383, 1987).

The sponsor submitted NDA 21-252 for FIV-ASA (mesalamine) suppositories for the treatment of active ulcerative proctitis. The application was filed under section 505 (b) (2). Mesalamine suppositories (Rowasa Suppositories, Solvay Pharmaceuticals) had been available in the US market since FDA approval of NDA 19-919 in 1990 and well accepted as an effective and safe medication for the treatment of ulcerative proctitis. However, due to unanticipated withdrawal of the NDA by Solvay, there has been an urgent need for a replacement product in the US market. The sponsor, Axcen Scandipharm Inc., has been marketing a mesalamine suppository in Canada since 1986. As part of the NDA, the sponsor has conducted three new preclinical toxicity studies with 5-ASA: a 14 day intra-rectal toxicity study with 5-ASA suppositories in rabbits, a 14-day intra-rectal toxicity study with 5-ASA suppositories in beagle dogs and an *in vitro* mouse lymphoma forward gene mutation assay with 5-ASA. In addition to the studies conducted by the sponsor, the preclinical pharmacology and toxicology studies with mesalamine, submitted as parts of NDA 19-618 (Rowasa Rectal Solution) and NDA 19-919 (Rowasa Suppositories), and all the published studies through April 2000 on mesalamine were consulted. In oral toxicity studies with 5-ASA in rats and dogs, the common target organ of toxicity was identified as the kidney. Renal toxicity has also been observed in rats in the 127-week carcinogenicity study with mesalamine. However, in the 14-day intra-rectal toxicity study with mesalamine suppositories in rabbits, no nephrotoxicity was observed at intra-rectal doses up to 800 mg/kg/day. In dogs, nephrotoxicity was observed in a female animal (of 3) at an intra-rectal dose of 166.6 mg/kg/day for 14 days. As the drug has

only limited absorption after intra-rectal administration as compared with oral dosing (the major site of absorption being the upper small intestine), there is minimal risk of systemic toxicity with mesalamine suppositories. In addition, the benefits and safety of mesalamine suppositories in the treatment of ulcerative proctitis have been well established and accepted. From a preclinical standpoint, mesalamine suppositories at the proposed doses, is not expected to have any serious adverse effects on the recipients.

APPEARS THIS WAY
ON ORIGINAL

RECOMMENDATION:

From the preclinical standpoint, the NDA application is approvable.

Sushanta Chakder, Ph.D.
Pharmacologist

Date

Comment:

Jasti B. Choudary, B.V.Sc., Ph.D.
Supervisory Pharmacologist, HFD-180

Date

cc:

Original NDA

HFD-180

HFD-181/CSO

HFD-180/Dr. Chakder

HFD-180/Dr. Choudary

HFD-345- Dr. Viswanathan

R/D Init.: by J. Choudary 11/24/00

SC/deg: 12/04/00

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