

Mucosal 5-aminosalicylic acid concentration inversely correlates with severity of colonic inflammation in patients with ulcerative colitis

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Abstract

Background and aim—The treatment of ulcerative colitis (UC) with 5-aminosalicylic acid (5-ASA) does not have the same therapeutic effect in all patients. We tested the hypothesis that the effectiveness of the drug is related to its mucosal concentration.

Patients—Twenty one UC patients receiving oral 5-ASA (2.4–3.2 g/day) were enrolled in the study. Four were also receiving topical treatment (2 g/day).

Methods—Six endoscopic biopsies were taken from the rectum for measurement of 5-ASA concentrations (ng/mg) by HPLC; soluble interleukin 2 receptor (sIL-2R) concentrations (U/ml) were measured by ELISA and histology. Endoscopic and histological appearance was graded on a four point scale (0–3). The Wilcoxon's rank test and Pearson's correlation coefficient were used for statistical analysis.

Results—Mucosal concentrations of 5-ASA were significantly higher ($p=0.03$) in patients with endoscopic scores of 0–1 compared with those with scores of 2–3 (16.1 (range 10.2–45) *v* 5.5 (3.5–17.4), respectively) and in patients with lower histological inflammation compared with those with more severe scores (17.4 (10.5–45) *v* 8.9 (3.5–17.2), respectively) ($p<0.01$). In contrast, mucosal sIL-2R concentrations were significantly lower in patients with slight endoscopic and histological lesions than in those with more severe disease. A significant inverse correlation ($r=-0.85$) was found between 5-ASA and sIL-2R mucosal concentrations ($p=0.00008$).

Conclusions—In patients with UC, in the same area of the intestinal tract, we found that the higher the 5-ASA mucosal concentrations, the lower the IL-2R levels and endoscopic and histological scores. We hypothesise that maintenance of high mucosal 5-ASA concentrations in all colonic segments could contribute to improve clinical outcome in UC patients.

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Keywords: ulcerative colitis; 5-aminosalicylic-acid; interleukin 2

Ulcerative colitis (UC) is a chronic inflammatory disease of unknown aetiology sustained by an uncontrolled immunoinflammatory re-

sponse. Clinical findings have shown that 5-aminosalicylic acid (5-ASA) is effective both in the treatment of active disease and in the prevention of recurrence^{1–4} but its precise mode of action and optimal dosage remain unknown.^{5–7} Several studies suggest that the drug acts locally and thus several pharmaceutical formulations have been designed to ensure delivery of 5-ASA into the large bowel, the site of inflammatory lesions. In fact, the drug does not gain access to the colonic mucosa through the systemic circulation but is taken up from the intestinal lumen. Differences in tablet delivery systems as well as in intestinal behaviour and colonic segmental transit time may lead to differences in drug availability at the colonic mucosal level^{8–11} and could explain the interindividual variability in effectiveness often observed with 5-ASA. In fact, in vivo studies have shown that the same oral dose does not always exert the same therapeutic effect and increasing the oral dose does not invariably provide additional therapeutic benefit to all patients.^{12–16} As a direct dose-effect relationship between 5-ASA and almost all of its therapeutic targets has been clearly demonstrated in vitro,^{17–20} it is likely that in vivo, the therapeutic effect of 5-ASA depends directly on the actual mucosal concentration obtained in a single patient in a given colonic tract.

To investigate this issue, attempts have been made to correlate endoscopic, histological, and mucosal immunological activities of colitis, detected in a given segment of the colon, with mucosal 5-ASA concentrations measured in the same part of the intestinal tract.

Materials and methods

PATIENTS

Twenty one patients (aged 36–68 years; 13 males, eight females) with UC were studied during a programmed follow up involving clinical, endoscopic, and histological assessment. The study included patients with mild to moderate colitic activity as well as those in remission. All patients were receiving oral 5-ASA formulations (2.4–3.2 g/day; Asacol, Bracco, Italy) and four were also receiving topical treatment (2 g/day). None had concomitant immunological, renal, or hepatic disorders or were receiving steroids, immunosuppressives, or antibiotics.

Clinical assessment of colitis was performed according to criteria modified from Lennard-

Abbreviations used in this paper: UC, ulcerative colitis; 5-ASA, 5-aminosalicylic acid; sIL-2R, soluble interleukin 2 receptor.

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Jones and colleagues.²¹ Patients with no more than two bowel movements per day and no other signs or symptoms of colitis were defined as in remission (score 0). Mild disease (score 1) included those cases with three to five bowel movements per day or other symptoms of colitis, including rectal bleeding, anorexia, or nausea. Moderate disease (score 3) included more than six, but less than 10, bowel movements per day, with or without rectal bleeding, anorexia, or nausea. Finally, severe disease (score 4) referred to 10 or more bowel movements per day with one or more of the following signs: abdominal tenderness, pulse rate >100 beats/min, fever (>37.5°C). The endoscopic appearance of colitis was graded (from 0 to 3) according to the presence of oedema, erythema, mucosal exudate, texture, and bleeding.²² The histological degree of inflammation was graded according to the criteria of Morson and Dawson on the following scale: 0 (normal), 1 (slightly active), 2 (moderately active), and 3 (very active).²³ Mucosal immunological activity was evaluated by measuring levels of soluble interleukin 2 receptor (sIL-2R).

Patients were prepared for colonoscopy using 3 litres of oral polyethylene glycol solution on the day before the examination, from 4 to 7 pm. This time was chosen to allow patients to take their oral and topical treatment as usual: the last enema of 5-ASA the night before the examination at 11.00 pm and the last tablets at 8.00 am, 2–3 hours before colonoscopy.

All colonoscopies were performed by the same endoscopist who recorded the endoscopic appearance of the rectal mucosa on a separate form. From the same intestinal area, biopsies for measurement of 5-ASA and sIL-2R levels, and conventional histology were taken. Two biopsies for 5-ASA and two for sIL-2R detection were weighed and immediately frozen at -80°C for later assay. Biopsies for histological investigation were processed as usual.

TISSUE ANALYSIS

To obtain supernatants, biopsies were treated as previously described.²⁴ Briefly, specimens were gently washed and placed in polypropylene tubes (Becton Dickinson Labware, California, USA) at 0°C at a concentration of 5 mg tissue/ml in 0.5% human albumin/RPMI 1640 (Gibco, Paisley, UK). The specimens were sonicated and supernatants collected by centrifugation (1800 *g*) for 10 minutes. Supernatant samples were aliquoted and stored at -80°C until used.

5-ASA

Mucosal concentrations of 5-ASA were measured using a high performance liquid chromatography method described previously.²⁵ Briefly, analyses were performed on a chromatographic apparatus (Waters, USA) which consisted of a Model 510 solvent delivery system, a Model U6K injector valve, and an electrochemical detector Coulochem (ESA, USA) Model 5100A, equipped with a

conditioning cell (Model 5021), an analytical cell (Model 5011), and connected to a Model 746 integrator. After thawing, the biopsy specimen was placed in tubes containing 2 ml of methanol with internal standard. After sonication the supernatants were collected and evaporated to dryness. Samples were reconstituted with 100 µl of mobile phase and aliquots of each sample (5 µl) were chromatographed on an analytical column (Erbasil S C18, 250×4.6 mm id, particle size 10 µM; Farmitalia, Carlo Erba, Italy). The mobile phase was a mixture of 0.01 M Na₂HPO₄ (pH 3.0) (containing 0.1 mM EDTA, 0.1 M citric acid, and 0.1 mM heptanesulphonic acid) and methanol (85:15, v/v) delivered at a flow rate of 1 ml/min. The standard curve was linear in the selected range with an interassay coefficient of variation of less than 4.6%. Quality control samples were also run on each day of sample analysis. The limit of detection for 5-ASA was 0.1 ng/mg at a signal to noise ratio of 5.

sIL-2R

Mucosal concentrations of sIL-2R were measured using an enzyme linked immunosorbent assay test kit (Innotest hIL-2Rs, Innogenetics, Belgium). This assay was based on the dual immunometric sandwich principle and was performed according to the manufacturer's instructions.

STATISTICAL ANALYSIS

Mucosal concentrations of 5-ASA and sIL-2R in patients with low endoscopic and histological activity (score 0–1) were compared with those with more severe disease (scores 2–3) using Wilcoxon's rank sum test for unpaired data. Pearson's correlation coefficient was used to correlate mucosal 5-ASA and sIL-2R concentrations.

Data are reported as median (range). 5-ASA concentrations are expressed as ng/mg of tissue and IL-2R as U/ml of supernatant.

Results

The clinical activity of colitis was mild in seven, moderate in five, and in remission in the remaining nine patients. Nine patients showed a rectal endoscopic picture of moderate colitis (score 2), eight showed minimal signs of inflammation (score 1) while the remaining four patients presented an endoscopic appearance of remission. None showed severe endoscopic signs of disease (score 3). The histological grade of rectal mucosal inflammation was very active (score 3) in two patients, moderately active (score 2) in 10, and slightly active (score 1) in eight. One patient had a normal histological assessment (score 0).

Median mucosal concentrations of 5-ASA were 15.9 ng/mg (range 3.5–45). Patients with slight endoscopic lesions showed significantly (*p*=0.03) higher concentrations of mucosal 5-ASA than those with a moderate endoscopic score of colitis (16.1 ng/mg (10.2–45) *v* 5.5 ng/mg (3.5–17.4), respectively) (fig 1). Similarly, significant differences (*p*<0.01) were found for mucosal concentrations of 5-ASA between patients with low histological scores

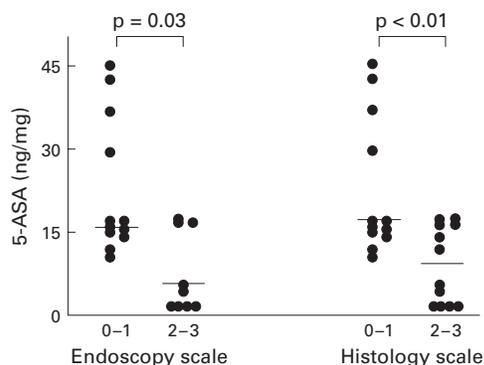


Figure 1 Distribution of 5-aminosalicylic acid (5-ASA) mucosal concentrations according to endoscopic and histological grading of colitis

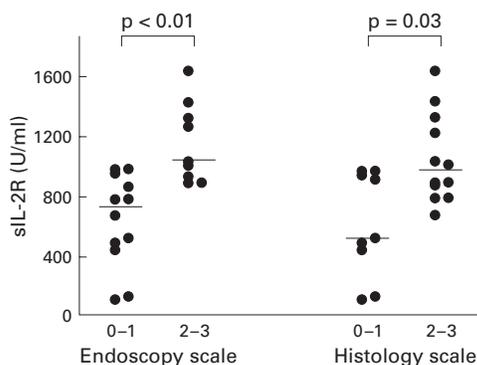


Figure 2 Distribution of soluble interleukin 2 receptor (sIL-2R) mucosal concentrations according to endoscopic and histological grading of colitis.

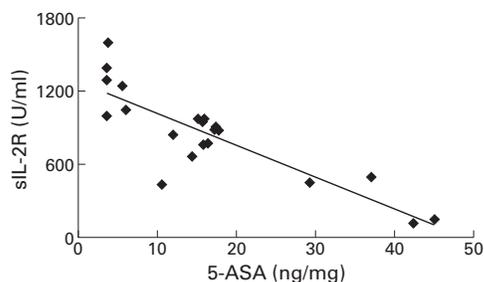


Figure 3 Correlation between mucosal concentrations of 5-aminosalicylic acid (5-ASA) and soluble interleukin 2 receptor (sIL-2R).

(score 0–1) and those with active histological inflammation (17.4 ng/mg (10.5–45) *v* 8.9 ng/mg (3.5–17.2), respectively) (fig 1).

Soluble IL-2R was detected in all supernatants. Median mucosal concentrations of sIL-2R were 890 U/ml (range 120–1600). Significantly ($p < 0.01$) lower levels of sIL-2R were present in patients with slight endoscopic disease compared with those with moderate endoscopic signs of disease (722 U/ml (120–975) *v* 1050 U/ml (890–1600), respectively) (fig 2). Similarly, significantly ($p = 0.03$) lower concentrations of sIL-2R were found in specimens with a low histological grade (score 0–1) compared with more inflamed specimens (500 U/ml (120–975) *v* 945 U/ml (670–1600), respectively) (fig 2).

There was a significant ($p < 0.001$) inverse correlation ($r = -0.85$) between mucosal concentrations of 5-ASA and sIL-2R (fig 3).

Discussion

Patients with UC chronically consume 5-ASA to reduce the frequency and severity of clinical recurrence. In spite of treatment, the clinical course of the disease is extremely variable, ranging from periods of prolonged remission to frequent episodes of relapse. Since one possible explanation for the high recurrence rate could be a poor response to treatment, a higher dosage of 5-ASA has been used to provide additional therapeutic benefit to patients. Clinical trials, however, failed to demonstrate a clear cut dose-response relationship for 5-ASA.^{12–16 26 27}

We have demonstrated an inverse relationship between mucosal concentrations of 5-ASA and UC disease activity—that is, in the same part of the intestinal tract, the higher the drug concentration, the lower the endoscopic and histological scores. Moreover, mucosal concentrations of 5-ASA were inversely correlated with mucosal levels of sIL-2R, a marker of mucosal inflammation.

In vitro studies have demonstrated that 5-ASA inhibits the activity of natural killer cells and the synthesis of inflammatory mediators (arachidonates, toxic reactive oxygen metabolites) and cytokines (interleukin 1, tumour necrosis factor α , interferon γ) in a dose dependent manner.^{28–35} In cell cultures, 5-ASA produced dose dependent inhibition of T cell proliferation by inhibiting IL-2 and IL-2R expression.^{36 37} In particular, in studies on isolated lamina propria mononuclear cells from patients with inflammatory bowel disease, Pullman and Doe demonstrated inhibition of IL-2 production by increasing 5-ASA concentrations from 0.1 to 2.5 mg/ml.³⁷

We have demonstrated for the first time in vivo that mucosal concentrations of 5-ASA are inversely related to those of sIL-2R, suggesting that the dose related effect of 5-ASA may depend on the concentration in the actual mucosa. Therefore, it is tempting to hypothesise that in patients with UC, if the drug does not reach a given therapeutic mucosal concentration its pharmacological effect is markedly reduced or absent. Indirect confirmation of this hypothesis is given by the effectiveness of 5-ASA enemas in patients with left colitis unresponsive to oral treatment.^{6 38 39} In fact, it has recently been demonstrated that topical treatment significantly increases mucosal drug concentrations obtained by oral treatment alone.⁴⁰

The relationship between 5-ASA oral treatment and mucosal concentrations of the drug has not been fully elucidated. Available data indicate that a given oral dose shows high interindividual variability in mucosal concentrations¹¹ and that increasing the oral dose does not lead to a corresponding increase in mucosal concentrations.⁴¹ Moreover, 5-ASA concentrates along the entire length of the colon in a heterogeneous fashion. In fact, after oral administration of 5-ASA, the highest concentrations have been detected in the right colon while in the rectum, the site invariably affected by the disease, the amounts were negligible.^{11 40} These findings indicate that the oral dose is not predictive of colonic distribu-

tion and concentrations of 5-ASA. If the relationship between tissue levels of the drug and disease activity emerging from the present investigation is confirmed, methods of obtaining mucosal concentrations of 5-ASA high enough to reach the mucosal therapeutic threshold of the drug should be investigated to offer UC patients more effective treatment.

However, the mucosal therapeutic range of 5-ASA is still unknown and cannot be established from the results of this study. Data emerging from in vitro studies fail to offer a solution to this problem as concentrations of 5-ASA that inhibit cytokines in experimental studies are much higher than the highest mucosal concentrations found in vivo.^{11 40 42 43} An explanation for this discrepancy could be that in in vitro studies the drug comes into contact with isolated cells, artificially activated by a given stimulus to produce different immunoinflammatory molecules whereas in vivo the drug acts and is distributed in a much more complex system, and inflammatory cells are stimulated in an uncontrolled way by unknown pathogens. Thus it is not surprising that data obtained in vitro do not fully correspond to those in vivo. Further studies to determine the mucosal therapeutic range of 5-ASA are warranted.

Mucosal concentrations of 5-ASA may be influenced by the severity of colonic inflammation. It is well known that colonic inflammation induces histological architectural distortion, epithelial damage, activation and collection of immunoinflammatory cells, and increased mucosal blood flow. These changes may be responsible for impaired mucosal uptake of 5-ASA. However, it is interesting that in two studies, moderate colitis did not impair either 5-ASA uptake or acetylation by colonocytes.^{44 45} Moreover, it has been demonstrated, by in vivo rectal dialysis, that the drug reduces colonic inflammatory mediators during the active phases of the disease.^{46 47} Finally, the widely recognised effectiveness of 5-ASA in treating active colitis may indicate that the drug is sufficiently absorbed and concentrated in the inflamed mucosa to exert its therapeutic effect.^{4 9 42} Thus even if no definitive conclusions can be made, indirect evidence seems to indicate that moderate inflammation does not play a major role in reducing mucosal concentrations of 5-ASA.

In conclusion, this study demonstrates that the endoscopic, histological, and immunological activities of UC are inversely related to mucosal concentrations of 5-ASA, thus suggesting that a poor response to 5-ASA treatment may be related to inadequate mucosal concentrations of the drug.

- 1 Azad-Khan AK, Howers DT, Pleits J, *et al.* Optimum dose of sulphasalazine for maintenance treatment in ulcerative colitis. *Gut* 1980;21:232-40.
- 2 Hanauer SB. Inflammatory bowel disease. *Drug Ther* 1996; 334:841-8.
- 3 Sachar DB. Maintenance therapy in ulcerative colitis and Crohn's disease. *J Clin Gastroenterol* 1995;20:117-22.
- 4 Sutherland LR, Roth DE, Beck PL. Alternative to sulfasalazine: a meta-analysis of 5-ASA in the treatment of ulcerative colitis. *Inflamm Bowel Dis* 1997;3:65-78.
- 5 Riley SA. What dose of 5-aminosalicylic acid (mesalazine) in ulcerative colitis? *Gut* 1998;42:761-3.

- 6 Kam L, Cohen H, Dooley C, *et al.* A comparison of mesalazine suspension enema and oral sulfasalazine for treatment of active distal ulcerative colitis in adults. *Am J Gastroenterol* 1996;91:1338-43.
- 7 Campieri M, Gionchetti P, Belluzzi A, *et al.* Optimum dosage of 5-aminosalicylic acid as rectal enemas in patients with active ulcerative colitis. *Gut* 1991;32:929-31.
- 8 Brodgen RN, Sorkin EM. Mesalazine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in chronic inflammatory bowel disease. *Drugs* 1989;38:500-23.
- 9 Mardini H AL, Lindsay DC, Deighton CM, *et al.* Effect of polymer coating on faecal recovery of ingested 5-aminosalicylic acid in patients with ulcerative colitis. *Gut* 1987;28:1084-9.
- 10 Laursen LS, Stockolm M, Bukhave K, *et al.* Disposition of 5-aminosalicylic acid by olsalazine and three mesalazine preparations in patients with ulcerative colitis. Comparison of intraluminal colonic concentrations, serum values, and urinary excretion. *Gut* 1990;31:1271-6.
- 11 DeVos M, Verdievel H, Schoonjas R, *et al.* Concentration of 5-ASA and Ac-5-ASA in human ileocolonic biopsy homogenates after oral 5-ASA preparations. *Gut* 1992;33: 1338-42.
- 12 Sutherland LR, May GR, Shaffer EA. Sulfasalazine revisited: a meta-analysis of 5-aminosalicylic acid in the treatment of ulcerative colitis. *Ann Intern Med* 1993;118: 540-9.
- 13 Ahluwalia NK, Mani V, Goodman MJ. Double-blind trial of 4.8g vs 2.4g of mesalazine for 4 weeks in the treatment of acute ulcerative colitis. *Gut* 1993;34:S11.
- 14 Travis SP, Tysk C, De Silva HJ. Optimal dose of olsalazine for maintaining ulcerative colitis remission. *Gut* 1994;35: 1282-6.
- 15 Kruijs W, Judmaier G, Kayasseh L, *et al.* Double-blind dose-finding study of olsalazine versus sulphasalazine as maintenance therapy for ulcerative colitis. *Eur J Gastroenterol Hepatol* 1995;7:991-6.
- 16 Mulder CJJ, van den Hazel SJ. Drug therapy: dose-response relationship of oral mesalazine in inflammatory bowel disease. *Med Inflamm* 1998;7:135-6.
- 17 Mahida YR, Lamming CED, Gallagher A, *et al.* 5-aminosalicylic acid is a potent inhibitor of IL-1 beta production in organ culture of colonic biopsy specimens from patients with inflammatory bowel disease. *Gut* 1996;32:50-4.
- 18 Reynolds PD, Middleton SJ, Shorthouse M, *et al.* The effects of aminosalicylic acid derivatives on nitric oxide in a cell-free system. *Aliment Pharmacol Ther* 1995;9:941-5.
- 19 Grisham MB, Ware K, Mashall S, *et al.* Prooxidant properties of 5-aminosalicylic acid. Possible mechanism for its adverse side effects. *Dig Dis Sci* 1992;37:1383-9.
- 20 Nielsen OH, Bouchelouche PN, Berild D, *et al.* Effect of 5-aminosalicylic acid and analogous substances on superoxide generation and intracellular free calcium in human neutrophilic granulocytes. *Scand J Gastroenterol* 1993;28: 527-32.
- 21 Lennard-Jones JE, Ritchie JK, Hidler W, *et al.* Assessment of severity in colitis: a preliminary study. *Gut* 1975;16:579-84.
- 22 McPhee MS, Swan JT, Biddle WL, *et al.* Proctocolitis unresponsive to conventional therapy response to 5-aminosalicylic acid enemas. *Dig Dis Sci* 1987;32:76-81S.
- 23 Morson BC, Dawson IMP. *Gastro-intestinal pathology*. Oxford: Blackwell, 1979:551-62.
- 24 Bryskov J, Tvede N, Andersen CB, *et al.* Increased concentration of interleukin-1beta, interleukin-2, and soluble interleukin-2 receptors in endoscopic mucosal biopsy specimens with active inflammatory bowel disease. *Gut* 1992;33:55-8.
- 25 Palumbo GC, Carlucci G, Mazzeo P, *et al.* Simultaneous determination of 5-aminosalicylic acid, acetyl-5-aminosalicylic acid and 2, 5-dihydroxybenzoic acid in endoscopic intestinal biopsy samples in humans by high-performance liquid chromatography with electrochemical detection. *J Pharmacol Biomed Anal* 1995;14:175-80.
- 26 Meyers S, Sachar DB, Present DH, *et al.* Olsalazine sodium in the treatment of ulcerative colitis among patients intolerant of sulfasalazine. *Gastroenterology* 1987;93:1255-62.
- 27 Schroeder KW, Tremaine WJ, Ilstrup MD. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. *N Engl J Med* 1987;317:1625-9.
- 28 Gibson PR, Jewell DP. Sulphasalazine and derivatives, natural killer activity and ulcerative colitis. *Clin Sci* 1985;69:177-84.
- 29 Aparicio-Pagès MN, Vespargat HW, Hafkenschid JCM, *et al.* Inhibition of cell mediated cytotoxicity by sulphasalazine: effect of in vivo treatment with 5-aminosalicylic acid and sulphasalazine on in vitro natural killer cell activity. *Gut* 1990;31:1030-2.
- 30 Allgayer H, Stenson WF. A comparison of effects of sulphasalazine and its metabolites on the metabolism of endogenous vs exogenous arachidonic acid. *Immunopharmacology* 1988;15:39-46.
- 31 Vespargat HW, Aparicio-Pagès MN, Verver S, *et al.* Influence of sulphasalazine and mesalazine on cellular and biochemical oxygen metabolite production. *Scand J Gastroenterol* 1991;26:779-86.
- 32 Greenfield SM, Hamblin AS, Shakoor ZS, *et al.* Inhibition of leucocyte adhesion molecule upregulation by tumor necrosis factor alpha: a novel mechanism of action of sulphasalazine. *Gut* 1993;34:252-6.

- 33 Bissonette EY, Enciso JA, Dean Befus AD. Inhibitory effects of sulphasalazine and its metabolites on histamine release and TNF- α production by mast cells. *J Immunol* 1996;156:218–23.
- 34 Kaiser GC, Yan F, Polk DB. Mesalamine blocks tumor necrosis factor growth inhibition and nuclear factor κ B activation in mouse colonocytes. *Gastroenterology* 1999;116:602–9.
- 35 Crotty B, Hoang P, Dalton HR, et al. Salicylates used in inflammatory bowel disease and colchicine impair interferon- γ induced HLA-DR expression. *Gut* 1992;33:59–64.
- 36 Stevens C, Lipman M, Fabry S, et al. 5-ASA abrogates T-cell proliferation by blocking interleukin-2 production in peripheral blood mononuclear cells. *J Pharmacol Exp Ther* 1995;272:399–406.
- 37 Pullman WE, Doe WF. IL-2 production by intestinal lamina propria cells in normal inflamed and cancer-bearing colons. *Clin Exp Immunol* 1992;88:132–7.
- 38 Biddle WL, Miner BP. Long-term use of mesalazine enemas to induce remission in ulcerative colitis. *Gastroenterology* 1990;99:113–18.
- 39 Farup PG, Hovde O, Halvorsen FA, et al. Mesalazine suppositories versus hydrocortisone foam in patients with distal ulcerative colitis. *Scand J Gastroenterol* 1995;30:164–70.
- 40 Frieri G, Palumbo G, Pimpo MT, et al. Rectal and colonic mesalazine concentration in ulcerative colitis: oral plus topical treatment. *Aliment Pharm Ther* 1999;13:1413–17.
- 41 Hussain F, Ajjan R, Trudgill N, et al. Dose loading with oral mesalazine—optimising drug concentrations in the mucosa. *Gastroenterology* 1996;110(suppl):A928.
- 42 Prakash A, Markham A. Oral delayed-release mesalazine. A review of its use in ulcerative colitis and Crohn's disease. *Drugs* 1999;57:383–408.
- 43 Frieri G, Pimpo MT, Andreoli A, et al. Prevention of post-operative recurrence of Crohn's disease requires adequate mucosal concentration of mesalazine. A GISC study. *Aliment Pharmacol Ther* 1999;13:577–82.
- 44 Allgayer H, Ahnfelt NO, Krus W, et al. Colonic N-acetylation of 5-aminosalicylic acid in inflammatory bowel disease. *Gastroenterology* 1989;87:38–41.
- 45 Ireland A, Priddle JD, Jewell DP. Acetylation of 5-aminosalicylic acid by isolated human colonic epithelial cells. *Clin Sci* 1990;78:105–11.
- 46 Lauritsen K, Laursen LS, Buckhave K, et al. Effects of topical 5-aminosalicylic acid and prednisolone on prostaglandin E2 and leukotriene B4 levels determined by equilibrium in-vivo dialysis of rectum in relapsing ulcerative colitis. *Gastroenterology* 1986;91:837.
- 47 Lauritsen K, Hansen J, Byster P, et al. Effects of sulphasalazine and disodium azodisalicylate on colonic PGE2 concentrations determined by equilibrium in-vivo dialysis of feces in patients with ulcerative colitis and healthy controls. *Gut* 1984;25:1271–8.