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Evaluation of renal function following treatment with 5-aminosalicylic acid derivatives in patients with ulcerative colitis

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SUMMARY

Background: A number of cases of nephrotoxicity have been reported in patients with inflammatory bowel disease taking oral 5-aminosalicylic acid (5-ASA).
Aim: To evaluate the effects of 9 months of therapy with mesalazine or olsalazine on renal function in patients with ulcerative colitis in remission.
Methods: Forty patients with ulcerative colitis in complete remission for 6 months were randomized to either olsalazine ($n = 20$) or mesalazine ($n = 20$ for nine months). Thirty-six of the 40 patients were on prior salicylate therapy. Disease activity was the measure of clinical efficacy and was assessed by the Harvey-Bradshaw Index (HBI). Laboratory efficacy measurements included glomerular filtration rate (GFR), microalbuminuria, urinary gluthathione S-transferase (GST) and serum C-reactive protein (CRP). Safety analysis consisted of documentation of adverse events and laboratory values.
Results: There was no significant reduction in the GFR overall on therapy. The levels of GFR adjusted for baseline were similar in the two treatment groups after 3, 6 and 9 months. A significantly higher percentage of mesalazine-treated patients experienced drug related adverse events, all of a minor nature. The incidence of adverse events causing early withdrawal was similar in the two treatment groups.
Conclusion: Treatment with mesalazine or olsalazine for 9 months had no significant impact on GFR.

INTRODUCTION

Sulfasalazine has been in use for almost four decades for the treatment of inflammatory bowel disease and consists of the salicylate, 5-aminosalicylic acid (5-ASA) and a sulpha moiety, sulfapyridine, joined by a diazo bond. The sulfapyridine moiety is now known to cause the majority of the adverse reactions of sulfasalazine,[1] whereas 5-ASA is the therapeutically active moiety.[2, 3] Oral sulfasalazine undergoes minimal metabolism or absorption in the upper gastrointestinal tract, whereas colonic bacteria degrade the diazo bond to release free 5-ASA.

Free 5-aminosalicylic acid cannot be given by mouth as it is unstable in acid and is rapidly absorbed by the small intestine.[4] Delivery systems have been developed to bypass this problem allowing intact 5-ASA to reach the distal small bowel and colon. Olsalazine consists of two 5-ASA molecules linked by an azo bond. It undergoes minimal small bowel absorption when given orally.[5] Like sulfasalazine, the azo bond of olsalazine is split in the colon, releasing both 5-ASA molecules to exert local therapeutic activity.[6] Several formulations of oral 5-ASA use pH-dependent polymer coatings designed to release 5-ASA to the target tissue. Asacol (mesalazine) is 5-ASA coated with Eudragit-S, an acrylic-based resin designed to dissolve at pH > 7[7]

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and can therefore deliver 5-ASA to the distal ileum and colon.[8] Furthermore, the pH in small intestine may vary, with intermittent periods of high pH.[9] Mesalazine is effective in the therapy of small bowel Crohn's disease,[10, 11] hence suggesting that release of this agent may occur in the small intestine.

Using 5-ASA in a pH coated preparation is typically minimally absorbed in its active form, most of it staying within the colon to be excreted in the faeces. The small amount of absorbed 5-ASA is acetylated by the liver and excreted in the urine. A portion of the 5-ASA that remains in the colon is rendered inactive within the gut lumen and within colonic epithelial cells by acetylation to N-acetyl-5-aminosalicylic acid (Ac-5-ASA) while the remaining portion stays as free 5-ASA.

Considerations of long-term safety of 5-ASA derivatives is of critical importance because lifelong maintenance treatment is usually recommended.[3] In rats short-term intravenous administration of free 5-aminosalicylic acid has been associated with nephrotoxicity.[12] Furthermore, numerous cases of nephrotoxicity have recently been described in inflammatory bowel disease patients who were on maintenance therapy with 5-ASA compound,[12–18] mesalazine in particular. Mesalazine related nephrotoxicity may be the result of systemic absorption of 5-ASA from the small intestine. However, renal damage has rarely been reported in patients treated with azo-cleaved forms of drug. Urinary excretion of olsalazine has been shown to be consistently less than 1% in patients with ileostomies, suggesting that almost 100% of this drug reaches the colon.[5] The mesalazine preparation used in comparison with olsalazine given in usual dosages, causes significantly higher levels of 5-ASA and Ac-5-ASA in the plasma and urine in patients with inflammatory bowel disease.[19, 20]

It is currently unclear whether nephrotoxicity is a accumulative dose-related or an idiosyncratic phenomenon in treatments with 5-ASA derivatives. However, nonsteroidal anti-inflammatory drugs (NSAIDs) have documented effects that may be dose-dependent or idiosyncratic.[21, 22]

An early sensitive indicator of glomerular damage would be a beneficial diagnostic aid in inflammatory bowel disease patients treated with 5-ASA. Recently, microalbuminuria has been reported to provide an accurate estimate of renal damage in patients with diabetes[23] and hypertension[24, 25] and could be an early marker of sub-clinical nephrotoxicity. In addition the study of Glutathione S-transferase (GST) excretion in urine may also be a sensitive indicator of renal tubular damage.[26]

This study was designed to evaluate renal function after continued exposure to either mesalazine or olsalazine in patients with ulcerative colitis who were in remission. The primary objective was to compare GFR, microalbuminuria and urinary GST activity in patients with ulcerative colitis during a 9-month treatment period with either mesalazine (Asacolon, 1.2 g/day) or olsalazine (Dipentum 1.0 g/day). The secondary objective was to examine whether microalbuminuria is a predictor of disease activity for ulcerative colitis and is influenced by treatment. The majority of patients (36/40) were maintained on salicylates prior to recruitment. Safety analyses were also conducted.

METHODS

Study design

This was a single centre, open, randomized, parallel group study of olsalazine vs. mesalazine in patients with ulcerative colitis in complete remission. Eligible patients were treated with either olsalazine or mesalazine for 9 months. The GFR was measured by Technetium 99m labelled diethylene triamine pentla-acidic (DTPA) scan; [Mallinckrodt] using a standard mathematical methodology.[27]

Clinical and laboratory assessments were performed before the start of treatment day (day 0), and after 3, 6 and 9 months of treatment. The simple index of Harvey and Bradshaw was used to monitor the clinical disease activity.[28] This is ordinarily used for Crohn's disease, however we have previously found that it correlates with objective markers of inflammation in ulcerative colitis.[29] Flexible sigmoidoscopy was performed to confirm endoscopic remission.[30] Patients who experienced a relapse of disease after inclusion remained in the study unless they were deteriorating clinically. Clinical relapse was confirmed with flexible sigmoidoscopy. The patients were monitored according to good clinical practice and given permissible treatment until clinical and sigmoidoscopic remission was obtained.

Inclusion criteria

Patients with a confirmed diagnosis of ulcerative colitis in remission for the last 6 months were recruited.

Remission was confirmed clinically using Harvey-Bradshaw Index (score < 3) and by a sigmoidoscopy (score of 0–1). Patients aged between 18 and 70 years were studied and all had written informed consent to participate in the study.

Exclusion criteria

Patients with known allergy to 5-ASA or other salicylates, pre-existing renal, hepatic, cardiac disease, diabetes mellitus or hypertension were excluded from the study. Patients with pregnancy, intended pregnancy or breast feeding were not recruited. Patients who received corticosteroid treatment in the past 6 weeks were excluded from the study. Patients receiving salicylates (dose > 300 mg/day), NSAIDs, immunosuppressives or cytotoxic drugs were also excluded from the study. Furthermore patients were excluded from the study who lacked the desire to participate in the study or those who participated in any other trial within the past month. Those patients who were using aspirin for cardiovascular disease or prophylaxis were also not recruited.

An institutional review board responsible for assuring the rights and safety of research patients prospectively reviewed and approved the protocol.

Treatment regimen

Study drug. Olsalazine (Dipentum; Pharmacia & Upjohn, Uppsala, Sweden) was provided as 250 mg capsules.

Reference drug. Mesalazine (Asacolon; PL-holder Shemour Ltd., Henlow, UK) was provided as 400 mg tablets coated by a 120-mm thick acrylic based resin (Eudragit-S) sensitive to pH.

Randomization and blinding

Patients received study medication according to a computer-generated randomization list. The code was not broken until all patients had completed the study. The analyses in serum and urine were performed on coded samples. The technitium (Tc) labelled diethylene triamine pentla-acidic (DPTA) scan was performed by a study-independent person to assess GFR.

Patients recruited in the study were randomized to maintenance treatment with either Dipentum (olsalazine) or Asacolon (mesalazine) for 9 months. Previous maintenance treatment was discontinued 1 day to initiation of therapy.

Dipentum was administered as two 250 mg capsules twice daily for a total daily dose of 1.0 g. Asacolon was taken as a single 400 mg tablet three times daily for a total daily dose of 1.2 g.

Concomitant therapy

Patients were allowed to take salicylic acid in therapeutic doses (up to 500 mg per day) or NSAIDs for 4 days or less during the study period. Salicylic acid or NSAIDs were not permitted within the 2 weeks prior to scanning. In case of disease relapse, 5-ASA enema and rectal or systemic steroids (< 20 mg daily) were permitted for a period not to exceed 3 weeks.

Efficacy assessments

At each visit the clinical disease activity was assessed with the Harvey-Bradshaw Index. The laboratory assessments were also made at the same visit.

Glomerular filtration rate (GFR). This was determined by DPTA scanning. Systemically injected technitium-labelled DPTA is primarily excreted by glomerular filtration and the calculation of GFR using this radiopharmaceutical is a commonly practised technique. Approximately 37 MBq of DTPA labelled Tc-99m was injected into the patient in a volume of 1 mL. The exact activity injected was determined by measuring the activity in the injection syringe both prior to and after the injection. This was carried out in a calibrated dose calibrator. The time of injection and the time of activity was measured and recorded. Concurrently a standard was also prepared. Blood samples were taken at 60, 120, 180 and in some cases 240 min. The exact time at which each of the samples were taken was recorded. The standard was then diluted to a volume of 1 litre. The activities in each of the blood samples and the standard were measured and the time of measurement noted. This was performed 6–8 h after injection. The measurement was performed using a sodium iodide detector connected to a Multi-Channel Analyser, this being peaked to Tc-99m. A background count was also obtained. The scan was performed at the same visit or within 1 week of the clinic visit.

Glutathione S transferase (GST). An immunoassay (Hepkit; Biotrin International) was used to measure GST levels in the urine.

Microalbumin. An immunoturbidimetric method (Microalbs; AMES, Bucks, UK) was used to measure microalbumin in urine.

C-reactive protein (CRP). CRP was measured by nephelometry using a commercially available ELISA (Biosource International, Camarillo, CA).

SAFETY ASSESSMENTS

At each visit the patient was asked the question 'Has treatment upset you in any way?'. The possible adverse events were recorded. In addition, venesection was performed at all clinic visits for haematology (haemoglobin, white cell count, platelet, haematocrit) and biochemistry (bilirubin, aspartate aminotransferase, alkaline phosphatase, gamma glutamyltransferase, urea, sodium, potassium, calcium). All patients also had urinalysis for protein or blood by dipstick method.

ADVERSE EVENTS

Patients were to be withdrawn from the study if it was deemed medically necessary by the investigator or it was the wish of the patient. A patient, once withdrawn was not re-included in the study. Patients were withdrawn from the study who developed intolerable adverse events or needed surgery. The other withdrawal criteria included pregnancy, patients desire and the clinical deterioration of the patient as determined by the investigator.

STATISTICAL ANALYSIS

The objective of this study was to compare the olsalazine and mesalazine groups with respect to efficacy and safety measures for patients with ulcerative colitis in confirmed remission. Efficacy end-points included GFR, GST and microalbuminuria. Safety end-points included medical events and other laboratory assays.

The baseline characteristics were investigated to determine whether the olsalazine and mesalazine groups were similar at entry to the study. For the demographic/baseline data, the analysis included only treatment in the model and was a comparison of the two treatment groups. Categorical data were analysed using a chi-squared test. Continous variables were analysed using one-way ANOVA. The primary efficacy assessment between the two treatment groups was carried out using one-way ANOVA of GFR and microalbumin at day 0, and month 3, 6 and 9.

The secondary efficacy assessment was an examination of the relationship of microalbumin and Harvey-Bradshaw Index within each treatment group. Because of the large number of zero values for both of these variables, this was done by means of frequency tables only. The number of patients in clinical remission was compared with chi-squared tests at each time point. Adverse events rates in the two treatment groups were compared using Fishers Exact test. The haematology and biochemistry laboratory assays at day 0, and months 3, 6 and 9 between the two treatment groups were compared by Kruskal–Wallis tests, and within each treatment group changes were tested using Wilcoxon Signed Rank tests. Modelling of the relationship of Harvey-Bradshaw index and microalbumin was not done due to the large number of zeroes. Similarly, modelling of the relationship of CRP and microalbumin was not done.

RESULTS

Forty patients with ulcerative colitis (male, $n = 22$; female, $n = 18$; age 43.2 ± 2.64 mean ± s.e.; range 18–70 years) in confirmed remission were recruited in the study (randomized to olsalazine, $n = 20$; mesalazine, $n = 20$); please see Table 1 for further demographic details. There was no statistically significant differences seen in the demographic or clinical variables in the two treatment groups. The olsalazine treated group was diagnosed with ulcerative colitis at a younger age (olsalazine 31.7 vs. mesalazine 36.2 years) compared to mesalazine treated group. The number of patients remaining in the study at each phase were identical between the two groups and 80% of the patients in each group completed the study (Figure 1).

Glomerular filtration rate (GFR) and microalbuminuria

The baseline GFR differed significantly between the patients randomized to mesalazine and olsalazine treated groups, when analysed according to dosed patients (mesalazine 81.8 mL/min vs. olsalazine 98.2 mL/min;

Table 1. The demographic details

Variable	Mesalazine ($n = 20$)	Olsalazine ($n = 20$)	P-Value
Age (mean [s.e.] years)	45.7 (2.93)	40.7 (2.35)	0.186
Gender			
Male	10 (50%)	12 (60%)	0.0525
Female			
Age at first diagnosis of ulcerative colitis (mean [s.e.] years)	36.2 (2.75)	31.7 (2.12)	0.197
Time since first diagnosis of ulcerative colitis (mean[s.e.] years)	9.44 (1.23)	9.06 (1.52)	0.847
Time in remission prior to treatment (mean [s.e.] months)	16.5 (3.13)	20.51 (5.52)	0.536
Maximum extent ever recorded			0.376
Proctitis (< 15 cm)	1 (5)	3 (15)	
Proctosigmoiditis	7 (35)	3 (15)	
Left sided	3 (15)	5 (25)	
Pancolitis	9 (45)	9 (45)	
Harvey-Bradshaw Index at day 0	0.3 (0.12)	0.1 (0.07)	0.294
Sigmoidoscopic findings			0.752
Grade 0	10 (50)	11 (55)	
Grade 1	10 (50)	9 (45)	
Current relapse preventing treatment			0.506
Mesalazine	1 (50	0 (0)	
Olsalazine	10 (50)	11 (55)	
Sulfasalazine	6 (30)	8 (40)	
None	3 (15)	1 (5)	
Other relapse preventing treatment (within last 2 year)	3 (15)	3 (15)	1.000

$P = 0.03$) but no significant difference was observed when analysed according to eligible patients. The GFRs at 3, 6 and 9 months did not differ significantly from the baseline in the two treatment groups (Figure 2). At 9 months GFR was significantly lower in the mesalazine treated group, however this can be explained by lower GFR at randomization compared with the olsalazine treated group. No significant difference was seen in microalbuminuria levels in the two treatment groups at 0, 3, 6 and 9 months (Table 2). The microalbuminuria levels exceeded the normal range (0–20 μg/min) in only two patients in the mesalazine-treated group (21.9 and 54.8 μg/min at 9 months) and one patient in the olsalazine treated group (51.0 μg/min at baseline). This finding is consistent with patients maintenance in remission, however the adjusted baseline microalbuminuria levels were significantly lower in the mesalazine treated group compared to olsalazine treated group (3.34 ± 1.29 vs. 7.84 ± 1.29 μg/min; $P = 0.024$).

Urinary GST activity remained with in the normal range in both groups throughout the study period (data not shown).

Clinical remission

The percentage of patients in clinical remission at 3, 6 and 9 months did not differ significantly between the mesalazine and olsalazine treated groups. Ninety per cent of patients in both groups were in remission at 9 months of treatment. One patient in the olsalazine treated group who suffered a relapse was found to have grade 4 sigmoidoscopic findings.

Safety evaluation

Safety evaluation were performed on all patients who received one dose of drug (either mesalazine or olsalazine). The percentages of patients remaining in the study

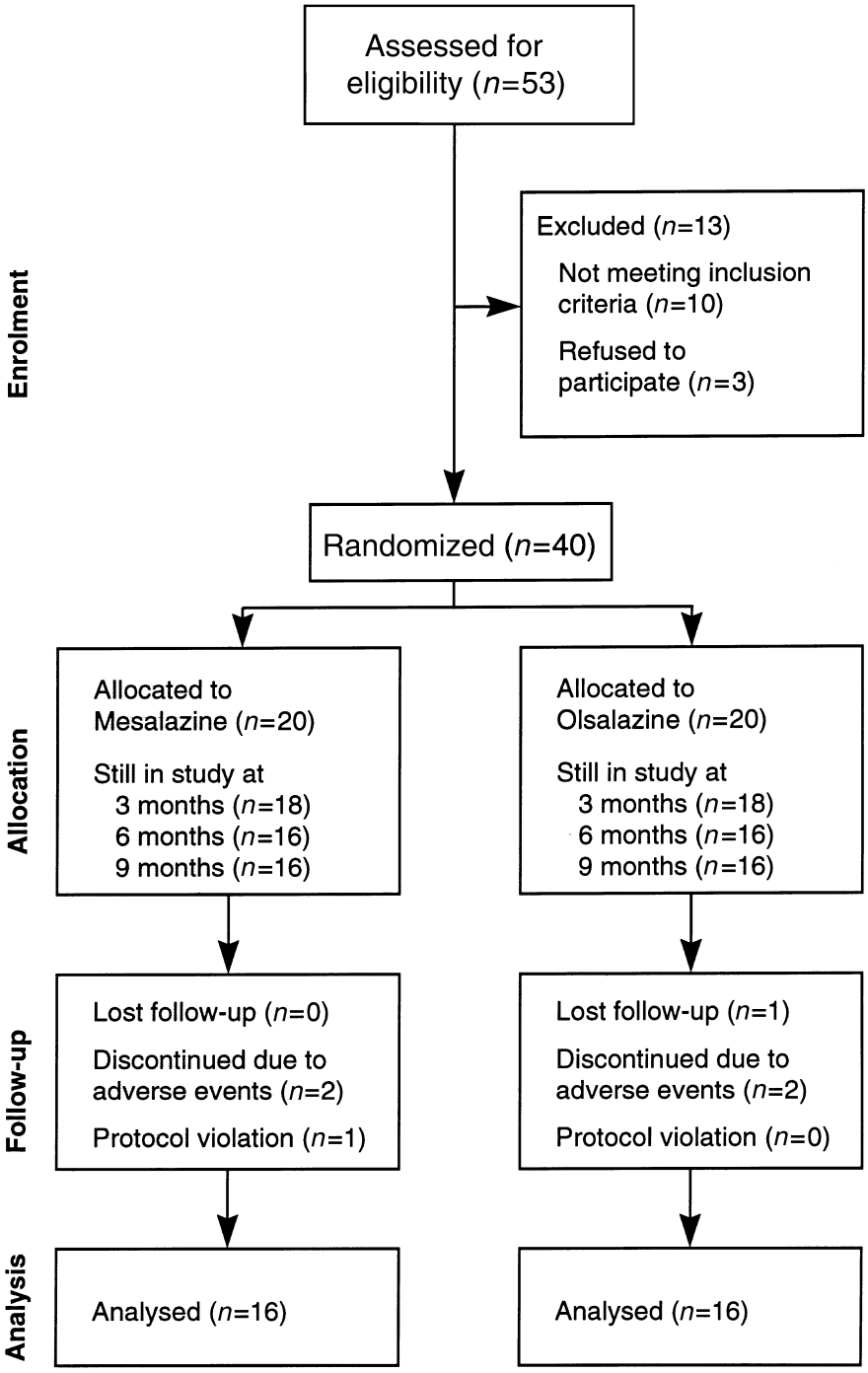

Figure 1. Flow diagram of the progress through the phases of a randomized trial.

at each phase were identical between the two treatment groups. The mean duration of dosing was similar in the two treatment groups (mesalazine 8.41 ± 0.51 vs. olsalazine 8.92 ± 0.29 months; $P = 0.90$). Compliance with study medication did not differ in the two treatment groups. At months 3, 6 and 9, the compliance rate among mesalazine treated patients was 100%, 100% and 80%, respectively, and among olsalazine treated patients was 100%, 94.1% and 95%, respectively.

Forty per cent (8/20) of mesalazine treated patients reported at least one adverse event with a total of 27 adverse events. However, 30% (6/20) in the olsalazine treated group reported adverse events (12 in total). All adverse events reported were mild to moderate in

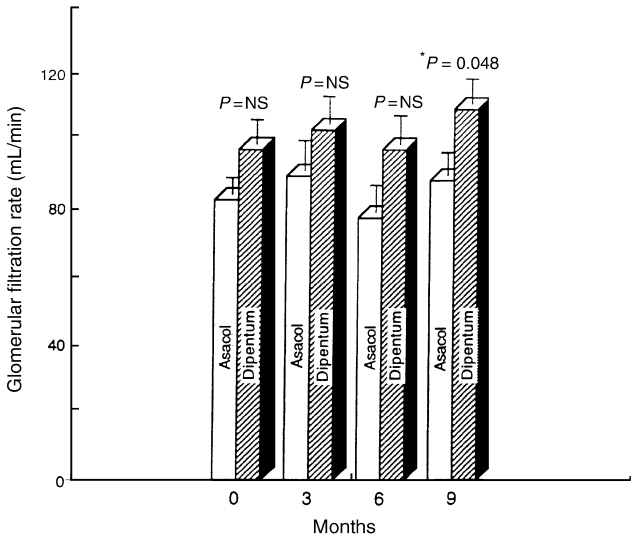

Figure 2. Comparison of glomerular filtration rate (GFR) in quiescent ulcerative colitis patients receiving maintenance therapy with Dipentum (olsalazine) vs. Asacol (mesalazine) at 0, 3, 6 and 9 months duration.

severity. No serious adverse events or death were reported in the two treatment groups. The most frequent categories of adverse events were 'body as a whole' category 20%. In this category the frequency of symptoms such as abdominal pain and abdominal distension were similar in the two treatment groups and digestive system related in 12.5% of patients. The frequency of adverse events classified as digestive system related, which included dyspepsia (2), nausea (2) and diarrhoea (1), were higher for mesalazine treated patients (mesalazine 25% [5/20] vs. olsalazine 0% [0/20]; $P = 0.047$).

Discontinuation of study due to adverse events

Adverse events led to the withdrawal of four patients, two patients from each treatment group. In mesalazine group a 54-year-old female and 48-year-old male were withdrawn from the study due to symptoms of abdominal pain, abdominal distension, dyspepsia and nausea. However, in olsalazine group a 40-year-old female patient was withdrawn from the study due to symptoms such as backache, generalized body aches and pains and insomnia. Another 48-year-old female patient was also withdrawn from the olsalazine group due to hypertension. The symptoms reported in the two treatment groups were considered to be of moderate severity.

No changes were seen from the baseline in routine haematological and biochemical parameters in the two treatment groups at 3, 6 and 9 months.

Table 2. Microalbuminuria (μg/min, mean ± s.e.); normal range (0–20 μg/min)

	Mesalazine	Olsalazine	*P*-value
Baseline	6.44 ± 1.7	5.58 ± 1.2	0.69
3 month	3.78 ± 1.3	7.13 ± 1.3	0.09
6 month	7.73 ± 1.7	3.61 ± 1.2	0.06
9 month	9.11 ± 4.7	3.84 ± 1.3	0.37

DISCUSSION

Recent case reports suggest that renal damage such as acute renal failure, acute nephritis or nephrotic syndrome may occur in patients receiving 5-ASA treatment.[13–18] Usually, cessation of 5-ASA drug therapy results in reversibility, however in some cases permanent clinical renal dysfunction has been reported.[16–18]

In this randomized study we evaluated renal function by studying glomerular filtration rate, microalbuminuria and urinary glutathione S-transferase over a 9-month period in patients with quiescent ulcerative colitis receiving treatment with either mesalazine or olsalazine.

The GFR at baseline and after 9 months of treatment was significantly lower in the mesalazine-treated group. However, the levels of GFR, adjusted for baseline were similar in the two treatment groups after 3, 6 and 9 months. Occasionally, patients in each treatment group had low GFR levels with no clinical or biochemical significance.

It has been suggested that intrarenal prostaglandin synthesis contributes to the maintenance of renal haemodynamics.[31] Prostaglandin inhibition by NSAIDs or NSAID-like medication may reduce GFR.[32] Wallis *et al.* have shown that the use of NSAIDs in arthritis patients reduces the effective renal plasma flow (ERFF), effective renal blood flow (ERBF) and GFR, although these events were not associated with untoward clinical effects.[33] Recent studies have demonstrated that inflammatory bowel disease patients treated with pH-dependent mesalazine preparations have significantly high levels of free 5-ASA and Ac-5-ASA in the plasma and urine compared to diazo bond 5-ASA preparations.[19, 20] Nevertheless, there is a theoretical possibility that high circulating 5-ASA levels may

impair the ability of the kidney to preserve GFR if a further stress were to supervene, such as acute relapse of inflammatory bowel disease or use of concomitant medication with a potential nephrotoxic property. In this study, however, neither mesalazine nor olsalazine in chronic treatment of ulcerative colitis during the remission period have an effect on GFR, microalbuminuria or urinary GST activity.

More recently, Schreiber *et al.* reported increased excretion of tubular protein in urine of patients with inflammatory bowel disease receiving more than 3 g of 5-ASA. They found a correlation between increased excretion of tubular proteins, alkaline phosphatase and γ-glutamyl transferase in urine and life-time dose of 5-ASA or sulfasalazine.[21] In another study, however, Kreisel *et al.* evaluated urinary enzymes such as β-N-acetyl-D-glucosaminidase (β-NAG), dipeptidylpeptidase-4 (DPP-4) and alanine aminopeptidase (AAP) as markers of renal tubular damage in patients with inflammatory bowel disease. High urinary β-NAG, DPP4 and AAP levels associated with active inflammation and normalization of these urinary enzymes was observed with successful medical therapy despite increasing cumulative doses of 5-aminosalicylic or sulfasalazine.[34] Similar markers studied by Riley *et al.* in patients with quiescent ulcerative colitis were also normal.[35] Although the exact mechanism of nephrotoxicity described in inflammatory bowel disease patients is not fully understood, there would appear to be at least two means of induction of renal disease. First, a hypersensitivity or idiosyncratic aetiology has been suggested that are independent of intake dose. Although, this overlooks the clustering of cases with pH dependent release preparations of 5-ASA suggesting the possibility of dose dependent cumulative mechanism, lacking symptoms and signs of hypersensitivity or idiosyncratic reactions.

Our study found no change in GFR over a 9-month period in patients with ulcerative colitis in remission treated with mesalazine or olsalazine. We believe that nephrotoxicity seen in inflammatory bowel disease treated with therapeutic doses of 5-ASA are probably idiosyncratic. However, larger long-term studies are needed to determine the exact mechanism of nephrotoxicity in inflammatory bowel disease.

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