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5-*amino salicylic acid absorption and metabolism in ulcerative colitis patients receiving maintenance sulphasalazine, olsalazine or mesalazine*

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SUMMARY

Background: All 5-aminosalicylic acid (5-ASA) preparations are potentially nephrotoxic, but there has been concern that newer delivery systems may increase this risk, either because of altered absorption or altered metabolism. Previous studies of 5-ASA absorption and excretion have usually either been performed in healthy controls or have only examined short-term therapy. 5-ASA and *N*-acetyl-5-ASA have therefore been measured in blood samples, and *N*-acetyl-5-ASA in urine samples, from patients with ulcerative colitis on long-term maintenance with different 5-ASA preparations and compared with sensitive markers of renal damage.

Methods: Patients receiving mesalazine (Asacol) ($n = 13$), sulphasalazine ($n = 12$) or olsalazine (Dipentum) ($n = 8$), all at doses within the recommended range were studied. Six-hour and trough serum concentrations of 5-ASA and *N*-acetyl-5-ASA and 24-h urinary excretion of *N*-acetyl-5-ASA were measured by high-performance liquid chromatography.

Results: Absorption of 5-ASA, assessed as 24-h excretion of *N*-acetyl-5-ASA expressed as molar % of ingested dose, was greater in patients receiving mesalazine, $23.25 \pm 10.65\%$ (mean $\pm$ s.d.; $n = 13$), than those receiving sulphasalazine ($11.16 \pm 10.52\%$, $n = 12$; $P = 0.003$) or olsalazine ($9.70 \pm 3.89\%$, $n = 8$; $P < 0.002$). The ratio of 5-ASA:*N*-acetyl-5-ASA in the serum 6 h after dose was also greater with mesalazine (1.02 ± 0.44, mean $\pm$ s.d.) than sulphasalazine (0.54 ± 0.44, $P < 0.02$) or olsalazine (0.38 ± 0.44, $P < 0.005$). Urinary markers of tubular damage were increased in four of 33 patients, but showed no correlation with concentration of 5-ASA or *N*-acetyl-5-ASA in serum and *N*-acetyl-5-ASA in urine, nor with lifetime dose or average daily dose of 5-ASA.

Conclusions: In patients with ulcerative colitis receiving maintenance 5-ASA therapy there was greater absorption and less acetylation of 5-ASA from mesalazine (Asacol) compared with sulphasalazine or olsalazine, but no evidence from this study that this resulted in increased nephrotoxicity.

INTRODUCTION

There have been considerable developments in 5-ASA delivery with the introduction of coated and dimerized preparations of 5-ASA such as mesalazine (Asacol; Smithkline and French, Brentford, UK) and olsalazine (Dipentum; Pharmacia, Uppsala, Sweden), respectively, resulting in avoidance of sulphapyridine-related side-effects. However, there has been anxiety regarding recent reports of nephrotoxicity with Eudragit-coated preparations. These reports include well-documented cases of biopsy proven interstitial nephritis[1] and nephrogenic diabetes insipidus.[2] Such cases led to the Committee on Safety of Medicines (UK) highlighting nephrotoxicity due

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to mesalazine in 1990.[3] Up until the end of June 1996, 95 renal reactions had been reported for mesalazine, including 30 cases of interstitial nephritis, out of a total of 907 reactions reported since January 1985. These figures include reports for all forms of mesalazine. For olsalazine, there have only been two renal reactions (one being interstitial nephritis) out of 102 reported reactions since 1987. For sulphasalazine, there have been 60 renal reactions out of 2314 reactions reported since 1964 (Committee on Safety of Medicines, personal communication). These have included two cases of biopsy proven interstitial nephritis.[4] It is uncertain whether this merely reflects increased reporting rates due to the recent introduction of new delivery systems or whether there is a genuine increased risk of nephrotoxicity due to altered 5-ASA uptake or metabolism.

Previous studies have assessed the relative absorption and excretion of 5-ASA from different 5-ASA drugs in short-term studies of patients with colitis, but no study has compared absorption and acetylation of 5-ASA in patients with ulcerative colitis receiving long-term maintenance therapy.[5–8]

The present study was aimed at determining whether ulcerative colitis patients receiving maintenance therapy with different 5-ASA preparations at conventional therapeutic dosage differed either in terms of 5-ASA absorption or acetylation, or in respect of renal damage determined using sensitive markers.

MATERIALS AND METHODS

Patients

Thirty-three patients with ulcerative colitis took part in the study. Patients were receiving maintenance therapy with sulphasalazine, mesalazine (Asacol) or olsalazine, all within the manufacturers recommended dosage ranges. Patients were under the care of two consultants; one consultant had been using mesalazine (Asacol) as first-line therapy ($n = 11$; total number of patients on mesalazine, $n = 13$) and the second consultant had been using sulphasalazine as first-line therapy ($n = 10$; total number of patients on sulphasalazine, $n = 12$). Olsalazine was generally used in cases of sulphasalazine intolerance ($n = 5$), although three patients had received olsalazine as initial therapy (total number of patients on olsalazine, $n = 8$). Diagnosis of ulcerative colitis had been established on clinical, histological and radiological criteria. Details of age, sex, drug therapy, duration of illness, stool characteristics and an assessment of disease activity were noted (see Table 1).[9] Each patient gave informed consent and the protocol for this study was approved by the Royal Liverpool University Hospital Ethical Committee.

Drug therapy

The daily doses of the 5-ASA preparations were: mesalazine 2.03 ± 0.65 g (mean $\pm$ s.d.); sulphasalazine 2.92 ± 0.6 g; and olsalazine 1.00 ± 0.93 g. Expressed in terms of moles of 5-ASA these doses correspond to 1.33×10^{-2}, 0.73×10^{-2} and 0.65×10^{-2} moles for mesalazine, sulphasalazine and olsalazine, respectively.

Experimental design

On the morning of the study patients omitted their normal dose of 5-ASA drug, and venous blood samples were taken (trough levels), centrifuged and serum stored at -80 °C. The samples were analysed for serum concentrations of creatinine and electrolytes, 5-ASA and *N*-acetyl-5-ASA. Patients then took their normal morning dose of 5-ASA preparation and a further serum sample was taken 6 h later. This sample, which should

Table 1. Demographic data for the patients studied

Patient group (n)	Median age (range)	Sex male:female	Duration of illness median (range) months	Disease activity inactive:mild:moderate	Prednisolone therapy n (dose range in mg)
Mesalazine (13)	47 (22–68)	7:6	24 (15–66)	8:3:2	$n = 5$ (2.5–30)
Sulphasalazine (12)	46 (29–69)	10:2	108 (42–204)	5:6:1	$n = 5$ (5–20)
Olsalazine (8)	43 (27–68)	5:3	37 (5–92)	5:0:3	$n = 2$ (5–10)

reflect the peak concentration,[5,6,10] was used to assess the ratio of unacetylated 5-ASA to *N*-acetyl-5-ASA. A 24-h urine sample was collected in a 2.5-L container containing 1 mL of 20% w/v chlorhexidine glucuronate solution as preservative. All patients were allowed food and drink *ad libitum* and took their normal dose of 5-ASA drug during this period.

The volume of the 24-h urine sample was measured and then stored at −80 °C to prevent degradation of analytes. Aliquots were analysed for *N*-acetyl-5-ASA, albumin and urinary markers of tubular damage (i.e. *β*-*N*-acetyl-glucosaminidase, transferrin, retinol-binding protein and IgG_4). Serum concentrations of 5-ASA and *N*-acetyl-5-ASA and 24-h urine excretion of *N*-acetyl-5-ASA were measured by high-performance liquid chromatography (HPLC). Urinary total protein, albumin, *β*-*N*-acetyl-glucosaminidase, transferrin, retinol-binding protein and IgG_4 were assayed as previously described and expressed per mmol creatinine excretion.[11]

HPLC analysis of 5-ASA and N*-acetyl-5-ASA*

HPLC analysis was performed using a Gilson 303 pump with an 802c manometric module, a Rheodyne 7125 injector and a Shimadzu SPD-2AS variable wavelength detector. The column used was Spherisorb ODS2 5 μm (Jones Chromatography Ltd, Glamorgan, UK) 250 × 4.6 mm, in conjunction with two guard columns in series: a Technicol pellicular pac (30 × 4.6 mm) and a Hypersil ODS 5 μm (30 × 4.6 mm). The mobile phase, 20% v/v methanol in 5 mM, pH 8.3, disodium hydrogen phosphate and 50 mM tetrabutylammonium chloride buffer (pH was adjusted with phosphoric acid), was sterile-filtered (0.45 μm), vacuum degassed and a column flow-rate of 1.7 mL/min was used. UV response was monitored at 254 nm, with a sensitivity of 0.01 or 0.005 absorbance units full scale (AUFS) for urine and serum analyses, respectively, and recorded on an LKB 2220 integrator. Standard 5-ASA and *N*-acetyl-5-ASA were kind gifts from Dr Stige Andersson (Pharmacia, Uppsala, Sweden). *p*-Aminosalicylic acid (4-ASA, internal standard) and tetrabutylammonium chloride (TBA) were purchased from Sigma Chemical Co. (Poole, Dorset, UK). HPLC-grade water, phosphoric acid and disodium hydrogen orthophosphate (AnalaR grade) were obtained from BDH Ltd (Poole, Dorset, UK). HPLC-grade methanol and acetonitrile were obtained from Fisons (Loughborough, Leicestershire, UK).

Stock solutions of 5-ASA, *N*-acetyl-5-ASA (both 100 μg/mL) and the internal standard 4-ASA (20 μg/mL) were prepared in mobile phase and stored in the dark at 4 °C. Control serum and urine samples were collected from six healthy volunteers on no medication. Standards were prepared in glass vials by adding appropriate volumes of stock solutions to 500 μL of control serum or diluted urine (1:4 with HPLC-grade water) to achieve final concentrations of 0.3125–5.0 μg/mL of 5-ASA and *N*-acetyl-5-ASA, and 1.25–30 μg/mL of *N*-acetyl-5-ASA, respectively. To this 250 μL of internal standard, 4-ASA (20 μg/mL), was added and it was then diluted to 1 mL with mobile phase. Blank serum or diluted urine (500 μL) samples were prepared by diluting with mobile phase to 1.0 mL.

Samples were vortexed thoroughly, centrifuged at 11 000 ***g*** for 20 min and aliquots of the supernatant (50 μL serum or 20 μL urine) injected onto the column. Similarly, 500-μL samples of unknown serum and diluted urine (range 1:4–1:24) were made up to 1.0 mL with 4-ASA internal standard (250 μL; 20 μg/mL) and mobile phase. All analyses were performed on blinded samples.

The ratio of peak height of 5-ASA or *N*-acetyl-5-ASA to that of the internal standard was determined. The regression line of peak height ratios vs. the standard concentrations of each metabolite was calculated by least-squares linear regression analysis. The concentration of 5-ASA and *N*-acetyl-5-ASA in the unknown samples was determined from the regression line.

Sensitivity and reproducibility of HPLC assay

Analysis of control serum spiked with 5-ASA and *N*-acetyl-5-ASA (0–5 μg/mL; 36 standards; $n = 6$ replicates) produced linear calibration curves with correlation coefficients (r) of 0.999. The within-assay coefficient of variation (CV) was 11.8% ($n = 6$) and repeated random analysis of standard-spiked serum samples throughout the analysis of unknowns showed good reproducibility with a between-assay CV of 11.9% ($n = 6$). The lower limit of detection for 5-ASA and *N*-acetyl-5-ASA in serum was 0.65 nmol/mL.

Assay of *N*-acetyl-5-ASA-spiked urine (0–30 μg/mL; 48 standards, $n = 6$ replicates) showed good linearity with increasing concentration ($r = 0.989$). The within-assay CV was 14.3% and between-assay CV was 15.4% ($n = 5$). The lower limit of detection for acetylated 5-ASA in urine was 5.6 nmol/mL. Unacetylated 5-ASA in urine could not be measured reliably by HPLC because of interference from other substances in urine.

Statistical analysis

Results were expressed as means ± standard deviation (s.d.) and statistical analysis was performed using Kruskall–Wallis analysis of variance (ANOVA) followed by two-tailed Mann–Whitney U-testing (ARCUS PROSTAT; Medical Computing; Aughton, Lancashire, UK). Differences were considered significant when $P < 0.05$.

RESULTS

The trough levels of 5-ASA were similar in patients receiving mesalazine (7.03 ± 11.14 nmol/mL, mean ± s.d., $n = 13$; $P = 0.22$), compared with those receiving sulphasalazine (2.03 ± 2.17, $n = 12$) or olsalazine (2.16 ± 2.39, $n = 8$) (see Figure 1). Although four of the patients taking mesalazine had particularly high trough 5-ASA levels.

The 6-h post-dose ratio of 5-ASA : *N*-acetyl-5-ASA was significantly higher in the mesalazine group (1.02 ± 0.44 nmol/mL, mean ± s.d., $n = 13$) when compared with the sulphasalazine (0.54 ± 0.44, $n = 12$; $P < 0.02$) and olsalazine (0.38 ± 0.19, $n = 8$; $P < 0.005$) groups (Figure 2). There was no correlation between the ratio of 5-ASA : *N*-acetyl-5-ASA and the molar dose of drug taken.

The urinary excretion of acetylated 5-ASA, expressed as a percentage of the dose delivered in moles, was significantly greater in the mesalazine group (23.25 ± 10.65%, mean ± s.d., $n = 13$) than in the sulphasalazine (11.16 ± 10.52%, $n = 12$; $P = 0.003$) and olsalazine (9.70 ± 3.89%, $n = 8$; $P < 0.002$) groups (see Figure 3).

There was no correlation between urinary β-*N*-acetyl-glucosaminidase (sensitive marker of proximal tubular toxicity) (Figure 4), transferrin (sensitive indicator of early glomerular damage), retinol-binding protein (excreted tubular protein), IgG_4, albumin or urinary total protein (see Figure 5) and average daily dose of drug or lifetime dose of drug. However, within each treatment

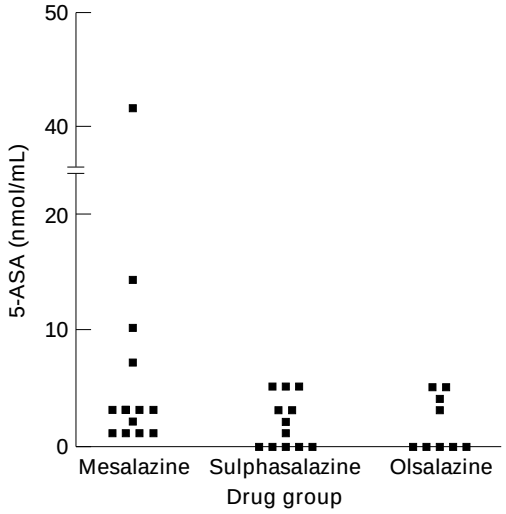

Figure 1. Trough serum 5-ASA levels according to drug therapy.

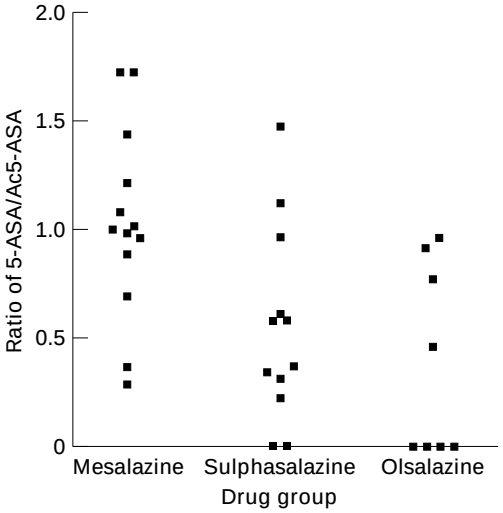

Figure 2. Six-hour post-dose ratio of serum 5-ASA : acetylated 5-ASA (Ac5-ASA) according to drug therapy.

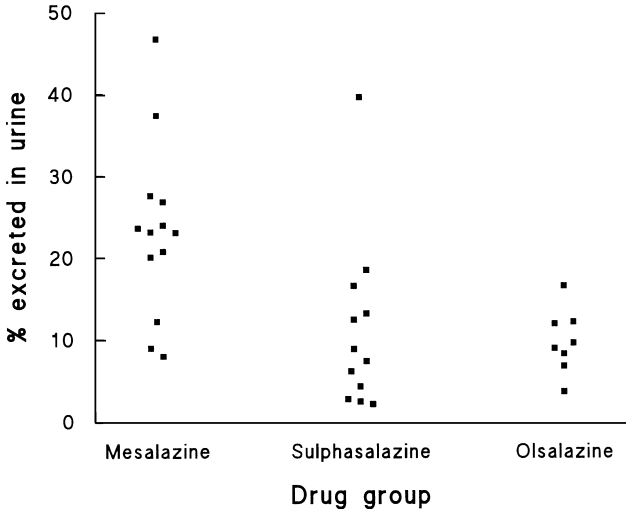

Figure 3. Absorption expressed as percentage molar dose excreted in the urine as acetylated 5-ASA (Ac5-ASA) according to drug therapy.

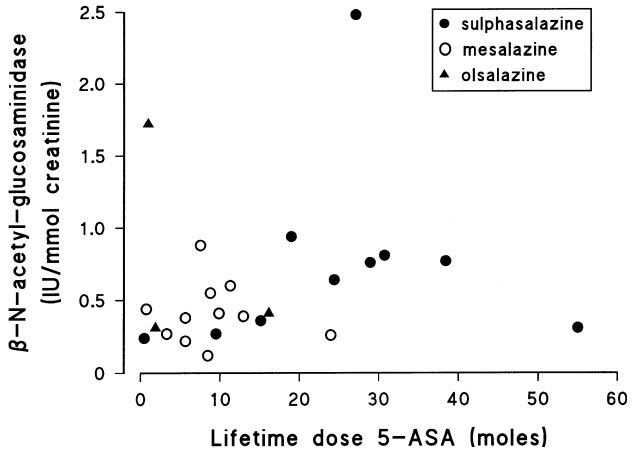

Figure 4. Urinary β-*N*-acetyl-glucosaminidase levels in relation to lifetime dose of 5-ASA compounds, according to drug therapy (only patients who had received a single 5-ASA preparation during their lifetime are shown; sulphasalazine $n = 10$, mesalazine $n = 11$, olsalazine $n = 3$).

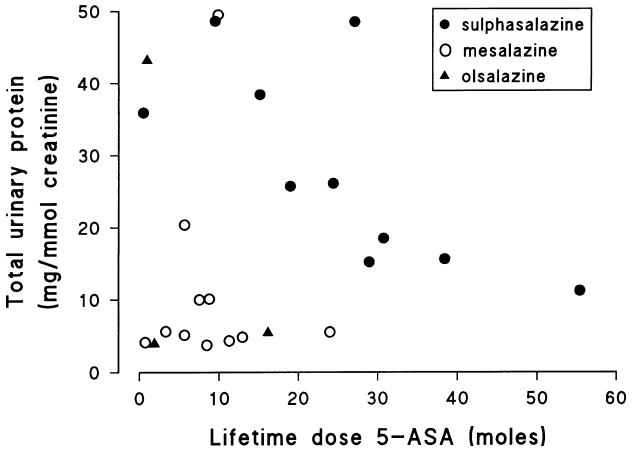

Figure 5. Urinary total protein levels in relation to lifetime dose of 5-ASA compounds, according to drug therapy (only patients who had received a single 5-ASA preparation during their lifetime are shown; as for Figure 4).

group there were patients with abnormal urinary excretion markers of renal damage when compared with upper reference levels derived from external controls; β-*N*-acetyl-glucosaminidase ($n = 2$), transferrin ($n = 3$), retinol-binding protein ($n = 1$), IgG_4 ($n = 1$) and albumin ($n = 3$).[10] These markers did not correlate with disease activity or disease duration. It is notable, that 17 of the 33 patients (10 sulphasalazine, three mesalazine and four olsalazine) had raised urinary total protein but, again, there was no correlation with disease activity or disease duration. Urinary total protein and transferrin excretion were, however, significantly greater in patients receiving sulphasalazine than mesalazine or olsalazine (Table 2).

DISCUSSION

This study demonstrates considerably greater absorption of 5-ASA from mesalazine (Asacol) than from olsalazine or sulphasalazine, and shows that a greater proportion of the 5-ASA in the blood is unacetylated in those patients receiving mesalazine. Although there is a suspicion that unacetylated 5-ASA may be more nephrotoxic than *N*-acetylated 5-ASA, there was no correlation between markers of renal damage and blood levels, average daily dose or total lifetime dose for any of the drugs.

The increased urinary excretion of 5-ASA from mesalazine, compared with sulphasalazine, confirms previous findings from short or single-dose studies.[5–8] Although urine unacetylated 5-ASA could not be measured in this study, previous work shows that 86% of excreted urine 5-ASA is in the *N*-acetylated form, so this probably reflects greater 5-ASA absorption.[12] Both this and the increased proportion of unacetylated 5-ASA in the

Table 2. Urinary markers of renal damage for each drug. Results are expressed as mean ± s.d. *$P < 0.005$, sulphasalazine compared with mesalazine; **$P < 0.02$, sulphasalazine compared with mesalazine

	5-ASA drug		
Marker	Sulphasalazine	Mesalazine	Olsalazine
β-*N*Ac-glucosaminidase (IU/mmol creatinine)	0.79 ± 0.64	0.39 ± 0.21	0.67 ± 0.63
Transferrin (mg/mmol creatinine)	0.31 ± 0.44*	0.13 ± 0.16	0.15 ± 0.16
Retinol-binding protein (μg/mmol creatinine)	9.69 ± 8.67	4.80 ± 2.56	6.60 ± 6.60
IgG_4 (mg/mmol creatinine)	9.47 ± 25.50	2.29 ± 2.04	0.82 ± 0.90
Albumin (mg/mmol creatinine)	3.18 ± 7.14	1.49 ± 3.17	2.90 ± 4.41
Total protein (mg/mmol creatinine)	28.37 ± 13.79**	12.33 ± 14.03	17.51 ± 22.31

mesalazine (Asacol) group may reflect the release of 5-ASA from mesalazine (Asacol) in the small intestine, either due to the slower small intestinal transit in ulcerative colitis or to pH-dependent 'dumping' in the small intestine.[13] The colon is more efficient at acetylating 5-ASA than the ileum, where the mucosal acetylation mechanism may be overwhelmed, leading to a greater proportion of the 5-ASA being in the unacetylated form.[14] Studies in both rabbits and humans suggest that the intestinal *N*-acetyl transferase is 'monomorphic', and is the main site of *N*-acetylation of 5-ASA,[14–16] unlike the polymorphic liver *N*-acetylase which is the site of acetylation after absorption.[17,18]

Association between drug levels and markers of renal damage cannot be studied using a short-term cross-over design and this study of patients continuing on their usual maintenance 5-ASA therapy seemed the most practical approach. The patients studied were broadly similar in terms of clinical activity and age. The olsalazine group was receiving a lower dose of 5-ASA, but the doses of mesalazine and sulphasalazine received correspond to conventional maintenance therapy. Moreover, there was no correlation between ratio of acetylated to non-acetylated 5-ASA and molar dose of mesalazine, so differences in dosing seem unlikely to explain the marked differences in metabolite concentration between patient groups.

The structural similarity between 5-ASA and phenacetin, together with the demonstration of necrosis of proximal convoluted tubules and renal papillary necrosis in rats treated with 5-ASA,[19] led to concern over the renal effects of 5-ASA-containing drugs. It has been shown that *N*-acetylation reduced the nephrotoxicity of phenacetin.[20] It has been inferred from this that the unacetylated 5-ASA is the potentially nephrotoxic form of the drug and that acetylation renders the drug safer, although this hypothesis has not been directly tested in animal models. The similarity with phenacetin might suggest that nephrotoxicity ought to be related to total life dose, but there is no clinical evidence to support this.

5-ASA-containing preparations are widely used for maintenance therapy in ulcerative colitis yet very few patients develop nephrotoxicity; which suggests that this might be an idiosyncratic reaction. However, this would seem not to be the case in several of the reported cases, as sulphasalazine had previously been well tolerated in the same patients.[1,2] In the present study a few patients had subtle evidence of nephrotoxicity, but this did not correlate with serum levels of 5-ASA, urine excretion, lifetime dose or average daily dose. These results are similar to a previous study investigating markers of renal damage in quiescent ulcerative colitis in patients taking sulphasalazine and mesalazine, where no difference was found between the groups, and no relationship found to either cumulative dose or duration of therapy.[21] However, high-dose mesalazine causes a minor rise in serum creatinine.[22] Markers of renal damage, such as microalbumin, have previously been reported to correlate with disease activity in inflammatory bowel disease, rather than with drug therapy.[23] It is notable that urinary protein excretion was significantly greater in the patients receiving sulphasalazine and that nephrotic syndrome has been reported on more occasions with this drug. This raises the possibility that the sulphapyridine component of the drug might have its own independent, and possibly different, nephrotoxic effect.

This study suggests that absorption is greater and acetylation proportionately less in patients receiving mesalazine (Asacol) compared with sulphasalazine or olsalazine, although there is no evidence from this study that this results in any increased risk of renal damage. However, a recent report in rats has shown that even a short-term systemic load of 5-ASA, in contrast with acetylated 5-ASA, dose-dependently induces renal glomerular and tubular damage suggesting that mesalazine formulations that cause high systemic exposures of 5-ASA in inflammatory bowel disease patients may have a potential risk of being nephrotoxic.[24] Therefore, at present, all 5-ASA preparations should be regarded as potentially nephrotoxic and appropriate monitoring of renal function performed on patients on maintenance therapy.

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