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Is *N*-acetylation of 5-aminosalicylic acid reversible in man?

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In two healthy male subjects the disposition of deuterated *N*-($^2H_3$) acetyl-5-aminosalicylic acid (d_3-ac-5-AS) was investigated after a single rectal dose of 500 mg d_3-ac-5-AS. Urine and plasma were analysed by h.p.l.c. and gas chromatography mass-spectrometry. Peak concentrations of around 0.5 μg/ml occurred within 6 h and plasma concentrations declined thereafter with a half-life ($t_{½}$) of about 6 h which was confirmed by urinary excretion data. Renal clearance of d_3-ac-5-AS ranged between 200 and 300 ml/min and only 4.4–11.2% of the dose could be recovered in the 48 h urine. Since no undeuterated ac-5-AS could be detected in any of the plasma and urine samples an irreversible acetylation of 5-AS is assumed in man.

Keywords 5-aminosalicylic acid acetylated metabolite deacetylation

Introduction

Several studies indicate that 5-aminosalicylic acid (5-AS) is the active moiety of sulphasalazine (SZ) in the treatment of Crohn's disease and ulcerative colitis (Khan *et al.*, 1977; Klotz *et al.*, 1980; van Hees *et al.*, 1980; Campieri *et al.*, 1981). 5-AS is liberated from SZ by bacterial azo cleavage of SZ in the colon and the absorbed 5-AS is mainly *N*-acetylated by capacity-limited gut wall and hepatic metabolism to *N*-acetyl-5-aminosalicylic acid (ac-5-AS) which is subsequently excreted into the urine (Das & Dubin, 1976; Pieniaszek & Bates, 1979; Fischer *et al.*, 1983). Fast and slow acetylators have not been described. However, there are conflicting data as to whether ac-5-AS might contribute to the therapeutic effect (Willoughby *et al.*, 1980; Binder *et al.*, 1981). These contradictory findings might be due to differences in study design and/or patient variability in drug disposition. Since acetylation is the major pathway for the elimination of 5-AS we investigated if an equilibrium exists between its acetylation and deacetylation to see whether reversible *N*-acetylation might contribute to the action of 5-AS. Such reversibility of acetylation has been observed with some sulphonamides (Schröder, 1973; Vree *et al.*, 1981, 1983) and might also occur with other acetylated drugs.

Methods

The synthesis and analysis of d_3-ac-5-AS (including d_0-ac-5-AS) in plasma and urine by h.p.l.c. and GC/MS have been described in detail elsewhere (Fischer *et al.*, 1981, 1984). The disposition and excretion of d_3-ac-5-AS was studied in two healthy male volunteers with normal kidney and liver function following a single rectal dose of 500 mg in the form of two suppositories. Blood samples (10 ml) were drawn after 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8 and 10 h. Urine was collected in fractions between 0–6 h, 6–12 h, 12–24 h and 24–48 h. Plasma and aliquots of urine were kept frozen (−25°C) until analysis.

The elimination half-life ($t_{½}$) of ac-5-AS was calculated from the log/linear decline of the plasma drug concentration (6–10 h postdosing) by linear regression analysis and from urinary excretion data by the rate and sigma-minus methods. The renal clearance (CL_R) of d_3-ac-5-AS was calculated from plasma and urine drug concentrations.

Results

Following rectal administration d_3-ac-5-AS was detected in the plasma of both subjects after 1 h

and its concentration peaked at around 0.5 μg/ml within 6 h. This was followed by a mono-exponential decline. The low plasma concentrations (range 0.05 to 0.5 μg/ml) suggested incomplete absorption and this was supported by cumulative urinary recoveries of 22.1 mg and 55.9 mg which accounted for 4.4 and 11.2% of the dose, respectively in each subject. The decline in plasma drug concentrations might have been influenced by a slow absorption process. The plasma $t_{½}$s of 6 h and 6.3 h for d_3-ac-5-AS were in reasonable agreement with values derived from the rate of urinary excretion (5.3 h and 9.4 h, respectively). The renal plasma clearance of ac-5-AS averaged 199 ml/min and 300 ml/min in the two subjects studied (Table 1).

GC/MS analysis of urine (1 day collection) and plasma samples (h.p.l.c. concentrations between 0.1 and 0.5 μg/ml) demonstrated in both subjects that the measured concentrations of ac-5-AS consisted only of d_3-ac-5-AS (Figure 1). No d_0-ac-5-AS was detected in any sample above the limit of background noise or natural content of d_3-ac-5-AS (purity ≥ 99.7%).

Discussion

There is conflicting information as to whether or not ac-5-AS has a therapeutic effect in the treatment of ulcerative colitis (Willoughby *et al.*, 1980; Binder *et al.*, 1981). Therefore, we investigated the possibility that acetylation of 5-AS might be reversible as a partial explanation of this discrepancy. The existence of such an acetylation-deacetylation equilibrium could be shown if a distinction can be made between ac-5-AS as administered drug and as apparent 'drug'- after deacetylation and reacetylation. The analytical problem can be resolved by using labelled ac-5-AS. Therefore, we used h.p.l.c. for the determination of total concentration of labelled and non-labelled ac-5-AS, whereas d_3-ac-5-AS was discriminated from ac-5-AS by GC/MS.

Since ac-5-AS was administered as retention enema in the studies of Willoughby *et al.* (1980) and Binder *et al.* (1981), we gave d_3-ac-5-AS in a similar form (suppositories). Owing to a limited availability of labelled ac-5-AS the supposed 'therapeutic' dose of 500 mg could be given only to two subjects. The GC/MS results (Figure 1) indicated that ac-5-AS is not deacetylated to a measurable extent. Similar results have been found with *N*-acetylprocainamide (NAPA) where only 2.8% of the administered NAPA-^{13}C was metabolized by deacetylation (Stec *et al.*, 1980). In contrast, the existence of a deacetylation-acetylation equilibrium in man has been demonstrated with a sulphonamide, N_4-acetyl-sulphamerazine (Vree *et al.*, 1983). The success in demonstrating such an equilibrium depends on the position of the equilibrium. However, in man acetylation of 5-AS is fast, as indicated by a short $t_{½}$ of 0.6 to 1.4 h (unpublished data), whereas elimination of ac-5-AS is relatively slow ($t_{½}$ 5–11 h, Table 1). Based on this pharmacokinetic comparison one may assume that the formation of ac-5-AS is rapid such that its renal clearance becomes the rate-limiting step in the overall elimination process of 5-AS. Similar rate limiting steps in the metabolite kinetics of 5-AS have been reported in rats (Houston & Cassidy, 1982). Since plasma ac-5-AS concentrations exceed those of 5-AS following multiple dosing with 5-AS (Fischer *et al.*, 1983) sufficient amounts of the metabolite would be available for deacetylation. However, the latter process could not be

Table 1 Disposition of d_3-ac-5-AS in two healthy subjects following rectal administration of 500 mg

	Subject 1 (60 kg, 37 years)	*Subject 2 (67 kg, 40 years)*
a: *Plasma data*		
Maximal concentration, (μg/ml)	0.45	0.55
Time of peak (h)	6	6
Apparent $t_{½}$ (h)	6.3	6.0
b: *Urine data*		
$t_{½}$ (h)	9.4[a]	5.3[a]
	11.4[b]	8.1[b]
CL_R (ml/min)	199	300
Ae (48 h) (mg)	55.9	22.1

[a]Calculated by the rate method
[b]Calculated by the sigma minus method

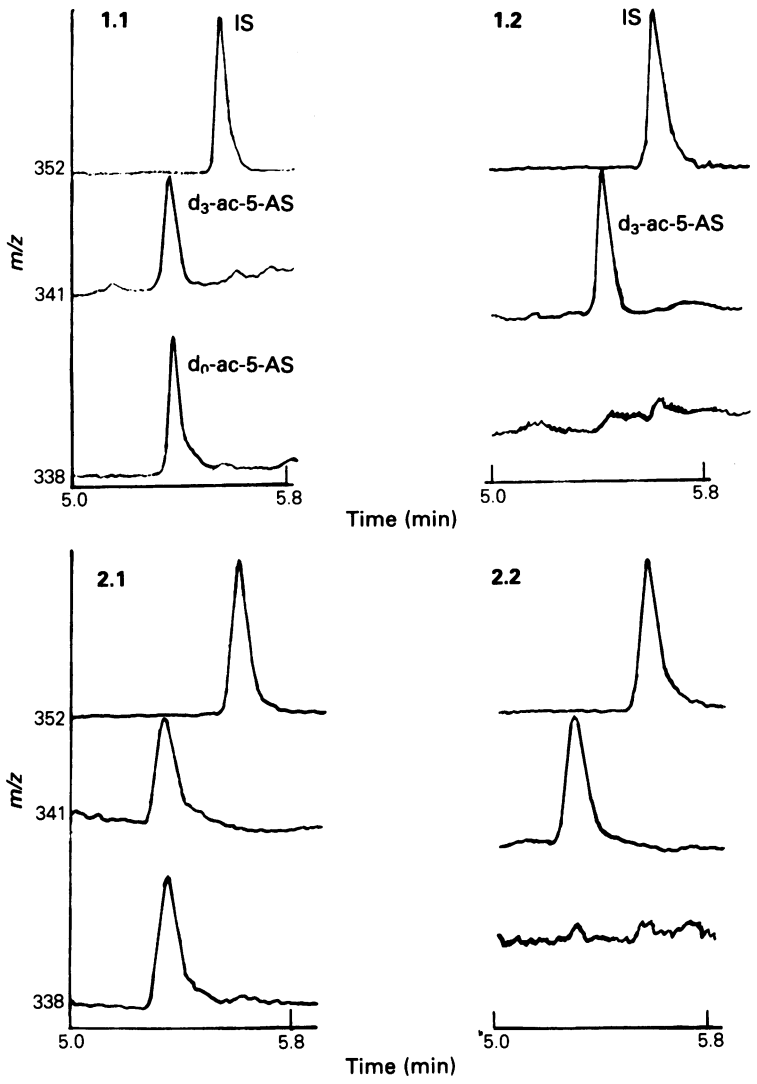

Figure 1 Typical GC/MS-chromatograms of plasma and urine extracts.
Electron impact mode 70 eV, selected ion monitoring m/z 352, 341 and 338 for the derivatives methylester- bis (trimethylsilyl) ether of internal standard, d_3-ac-5-AS and d_0-ac-5-AS, respectively.
1.1 Calibration: blank plasma spiked with non-deuterated and deuterated ac-5-AS (100 ng of each/1 ml diluted plasma).
1.2 Plasma extract from one of the volunteers at 6 h after drug administration.
2.1 Calibration: blank urine spiked with non-deuterated and deuterated ac-5-AS (100 ng of each/1 ml diluted urine)
2.2 Urine extract from one of the volunteers, 0–6 h urine collection after drug administration.
To each of the samples 400 ng of propionyl-5-AS as internal standard (IS) was added prior to analysis.

verified by our d_0/d_3-ac-5-AS measurements. Therefore, although we have studied only two individuals, it appears that the discrepancy in the results of investigations of the therapeutic efficacy of ac-5-AS (Willoughby *et al.*, 1980; Binder *et al.*, 1981) may not be explained by a metabolic phenomenon.

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References

Binder, V., Halskov, S., Hvidberg, E., Kristensen, E. Riis, P., Tougaard, L. & Willumsen, L. (1981). A controlled study of 5-acetaminosalicylic acid (5-Ac-ASA) as enema in ulcerative colitis. *Scand. J. Gastroenterol.*, **16**, 1122.

Campieri, M., Lanfranchi, G. A., Bazzochi, G., Brignola, C., Corazza, G., Cortini, C., Michelini, M. & Labo, G. (1981). Treatment of ulcerative colitis with high-dose 5-aminosalicylic acid enemas. *Lancet*, **ii**, 270–271.

Das, K. M. & Dubin, R. (1976). Clinical pharmacokinetics of sulphasalazine. *Clin. Pharmacokin.*, **1**, 406–425.

Fischer, C., Maier, K. & Klotz, U. (1981). Simplified high performance liquid chromatographic method for 5-aminosalicylic acid in plasma and urine. *J. Chromatogr.*, **225**, 498–503.

Fischer, C., Maier, K., Stumpf, E., von Gaisberg, U. & Klotz, U. (1983). Disposition of 5-aminosalicylic acid, the active metabolite of sulphasalazine, in man. *Eur. J. clin. Pharmac.*, **25**, 511–515.

Fischer, C., Meese, C. O. & Klotz, U. (1984). A stable isotope method for the quantification in plasma and urine of *N*-acetyl-5-aminosalicylic acid. *Biomed Mass Spectrom* (in press).

Houston, J. B. & Cassidy, M. K. (1982). Rate limiting steps in metabolite kinetics: formation of 5-acetaminosalicylate after administration of 5-aminosalicylate. *J. Pharm. Pharmac.*, **34**, 536–538.

Khan, A. K., Piris, J. & Truelove, S. C. (1977). An experiment to determine the active therapeutic moiety of sulphasalazine. *Lancet*, **ii**, 892–895.

Klotz, U., Maier, K., Fischer, C. & Heinkel, K. (1980). Therapeutic efficacy of sulfasalazine and its metabolites in patients with ulcerative colitis and Crohn's disease. *New Engl. J. Med.*, **303**, 1499–1502.

Pieniaszek, H. J. & Bates, T. R. (1979). Capacity-limited gut wall metabolism of 5-aminosalicylic acid, a therapeutically active metabolite of sulfasalazine, in rats. *J. pharm. Sci.*, **68**, 1323–1325.

Schröder, H. (1973). Deacetylation of acetyl sulphapyridine in man. *J. Pharm. Pharmac.*, **25**, 591–592.

Stec, G. P., Ruo, T. I., Thenot, J-P., Atkinson, Jr. A. G., Morita, Y. & Lertora, J. J. L. (1980). Kinetics of *N*-acetylprocainamide. *Clin. Pharmac. Ther.*, **28**, 659–666.

van Hees, P. A. M., Bakker, J. H. & van Tongeren, J. H. M. (1980). Effect of sulphapyridine, 5-aminosalicylic acid, and placebo in patients with idiopathic proctitis: a study to determine the active therapeutic moiety of sulphasalazine. *Gut*, **21**, 632–635.

Vree, T. B., Hekster, C. A., Baakman, M., Baars, I., Janssen, T., Oosterbaan, M., Termond, E. & Tijhuis, M. (1981). Rate constants of acetylation and deacetylation of some sulfonamide derivatives. *Current Chemotherapy and Immunotherapy*, Proceedings 12th International Congress of Chemotherapy, Florence, pp 140–142.

Vree, T. B., Tijhuis, M. W., Baakman, M. & Hekster, C. A. (1983). Analysis of N_4-trideutero-acetylsulphamerazine and its metabolites sulphamerazine and N_4-acetylsulphamerazine in man by means of high-performance liquid chromatography and mass spectrometry. *Biomed. Mass Spectrom.*, **10**, 114–119.

Willoughby, C. P., Piris, J. & Truelove, S. C. (1980). The effect of topical *N*-acetyl-5-aminosalicylic acid. *Scand. J. Gastroenterol.*, **15**, 715–719.

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