SPECIFIC MEASUREMENT OF 5-AMINOSALICYLIC ACID AND ITS ACETYLATED METABOLITE IN HUMAN BILE

5-Aminosalicylic acid (5-AS) appears to be the therapeutically active moiety of salicylazosulphapyridine (SASP, sulphasalazine), a drug of choice in the treatment of inflammatory bowel disease (Campieri *et al.*, 1981; van Hees *et al.*, 1980; Azad Khan *et al.*, 1977; Klotz *et al.*, 1980). SASP is split in the colon by bacteria into sulphapyridine (SP) and 5-AS. Subsequently, 5-AS is acetylated to the major metabolite *N*-acetyl-5-aminosalicylic acid (AcAS).

Recently, Azad Khan *et al.* (1982) investigated the disposition and metabolism of SASP in man by measuring urinary and biliary excretion of SASP and SP. However, no data could be presented for 5-AS in plasma and bile because of lack of a specific and sensitive assay devoid of interferences by exogenous and endogenous compounds.

We present here a highly specific method for the measurement of 5-AS and its acetylated metabolite in human bile. The work-up procedure is similar to the one for plasma and urine (Fischer *et al.*, 1981) but additional purification steps are mandatory to eliminate disturbing peaks from endogenous compounds before high performance liquid chromatographic (h.p.l.c.) separation can be applied.

The procedure of sample preparation is the following: 50–250 μ l bile in duplicates is made up to 1 ml with water and spiked with 20 μ l internal standard (50 μ g/ml *N*-acetyl-*p*-aminosalicylic acid, AcPAS). One of the duplicates is treated with 50 μ l acetic acid anhydride and shaken for 15 min.

In the first extraction the samples are acidified with 0.5 ml 1 *N* HCl and extracted into 8 ml diethyl ether. The evaporated residue is redissolved in 2 ml 0.067 *M* phosphate buffer (pH 7.4).

The aqueous samples are applied to Sep-Pak C-18 cartridges (Waters, Assoc., Milford, USA), washed with 3 ml 0.1 *M* phosphate buffer pH 7.4 and eluted with 3 ml of a mixture of water: methanol 75:25 (v/v).

In the second extraction step the water-methanol phase is acidified with 1 ml 1 *N* HCl and extracted twice with 5 ml diethyl ether. The dry residue is dissolved in 150 μ l mobile phase.

In the acetylation process with acetic acid anhydride 5-AS is quantitatively derivatized to AcAS and extracted and measured in this form. AcAS is more lipophilic than 5-AS which is difficult to extract into organic solvents. In addition, the much better fluorescence properties of AcAS allow a more sensitive measurement than 5-AS. The non-acetylated samples contain only the endogenous (or added) acetylated metabolite, whereas the difference between the acetylated and the non-acetylated duplicates results in the 5-AS concentration.

H.p.l.c. of 100 μ l aliquots was performed on a reversed phase column (Shandon ODS Hypersil 5

μ m, 4.6×120 mm) and eluted isocratically by a ternary solvent system (1.6 ml/min): 5% acetonitrile, 10% methanol and 85% 0.1 *M* phosphate buffer pH 2.5 (v/v). The eluate is monitored by a fluorescence detector (Spectra Physics FS 970, Darmstadt), excitation 300 nm, emission 430 nm. In Figure 1 typical traces are illustrated showing sharp and well separated peaks.

Figure 1 Typical traces of bile samples after h.p.l.c. separation (a) Blank bile, 250 μ l, (b) Blank bile, spiked with 5-AS and AcAS, 0.1 μ g/ml each, with internal standard, without acetylation, (c) Samples 1b following acetylation procedure and (d) Patient's bile, 12 h after administration of 1 g 5-AS.

Recoveries in the linear concentration range of 0.1 to 2.0 μ g/ml tested were 34–45% for AcAS ($n = 5$) and 44–53% for internal standard ($n = 5$). The coefficient of variation of the assay measured at three different concentrations was 4.8%.

Table 1 Total amounts of 5-AS and AcAS in human bile following a single dose of 1 g 5-AS rectally (A) or 2 g SASP orally (B) (= 0.76 g 5-AS) to seven patients.

	5-AS (mg/24 h)		AcAS (mg/24 h)		Collected bile (ml/24 h)		% recovered as total AS	
	A	B	A	B	A	B	A	B
Median	0.001	0.042	0.85	0.46	111	390	0.09	0.08
Range	0–0.03	0–0.028	0.21–6.12	0.05–0.91	85–380	87–500	0.02–0.61	0.01–0.12

We applied this method for the measurement of 5-AS and its major metabolite in human bile obtained from seven patients with biliary T-tube. Each patient received 5 days postsurgery in random order a single rectal dose of 1 g 5-AS or 2 g SASP orally. Bile was collected for 24 h after drug administration. The results are given in Table 1. Biliary recovery of total AS (5-AS and AcAS) ranged between 0.01 to 0.61% of the given dose. This will be an underestimation because bile collection was not quantitative, as judged from 24 h bile volumes sampled by the T-tube technique. Based on a normal bile flow of 1.2 l/day biliary excretion of total AS would be about 1%. As the bioavailability of the administered 5-AS is only 15% a biliary recirculation of approximately 7% of the absorbed dose can be assumed. Since 5-AS and

AcAS could be detected in human bile a minimal enterohepatic recirculation is likely.

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