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Note

High-performance liquid chromatographic determination of aminosalicylate, sulfapyridine and their metabolites

Its application for pharmacokinetic studies with salicylazosulfapyridine in man

C. FISCHER and U. KLOTZ

Dr. Margarete Fischer-Bosch Institut für Klinische Pharmakologie, Auerbachstrasse 112, D-700 Stuttgart-50 (G.F.R.)

Salicylazosulfapyridine (SASP; trade name Azulfidine from Pharmacia, Uppsala, Sweden) is the drug of first choice for the treatment of Crohn's disease and ulcerative colitis [1, 2]. It is split in the colon by gut bacteria to its two biologically active moieties aminosalicylate (AS) and sulfapyridine (SP). AS is eliminated mainly in acetylated form (AcAS). SP is metabolised to its N-acetyl-(AcSP) and 5-hydroxyl-(SPOH) derivate, and these two major metabolites are conjugated to their corresponding glucuronides. The acetylation rate is genetically controlled [3] and plasma concentrations of 20—50 μg total SP per ml are regarded as therapeutic (for review see ref. 4).

While this metabolism has been thoroughly investigated [5, 6], the pharmacokinetic properties of the drugs are almost unknown. Therefore we have developed specific and sensitive high-performance liquid chromatographic (HPLC) assays for the determination of AS, SP and their corresponding metabolites and studied the pharmacokinetics of these drugs in 7 healthy volunteers and 4 patients with Morbus Crohn or ulcerative colitis. In addition, since the reported side effects were associated with high plasma levels and mainly directed to slow acetylators, we also monitored these concentrations and phenotypes in 65 patients.

EXPERIMENTAL

The determination of SP and its metabolites has recently been described [7]. In Fig. 1 the extraction procedure for the determination of AS and AcAS is

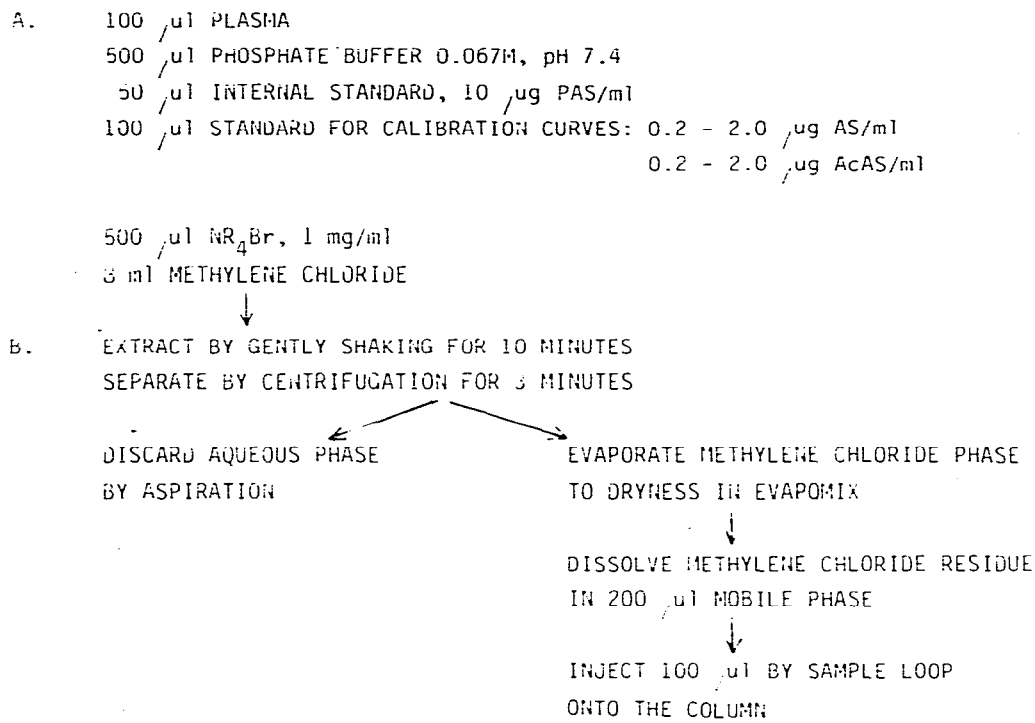

Fig. 1. Scheme of the extraction procedure for the determination of AS, its acetylated metabolite, AcAS, and the added internal standard, PAS.

outlined. Plasma (100 µl), the added internal standard *p*-aminosalicylate (PAS) and trimethylcetylammoniumbromide (NR_4Br) were extracted by methylene chloride. The evaporated residue of the organic phase was redissolved in 200 µl of the mobile phase and injected on to a reversed-phase column (250 × 3 mm; Nucleosil RP-18; particle size 10 µm). The mobile phase consisted of 50% methanol containing 1 g/l N,N,N,-trimethylcetylammoniumbromide. To achieve the sensitivity necessary to measure the low plasma levels of the salicylates a fluorescence-monitor had to be used (excitation 310 nm, emission 430 nm).

The separation of the three compounds was performed within 45 min by a flow of 1.3 ml/min. In the blank plasma, even if amplified ten-fold, no interfering peaks were observed (Fig. 2). If adding known amounts, AS was eluted first, close to the internal standard (PAS). The best sensitivity (factor 10) demonstrated AcAS (third peak). Since the unchanged AS had the most disadvantageous fluorometric spectrum, its peak was relatively small in the patient samples.

The pharmacokinetics of SASP was evaluated from single dose cross-over experiments (4 g SASP and 1.25 g SP one week apart) in 7 healthy volunteers and from steady state studies in 4 patients with Morbus Crohn or ulcerative colitis. Multiple blood samples (10 ml) were drawn for 65 and 36 h, respectively, following the single oral dose. During the steady state 8 specimens were collected following the last dose (3 g SASP/die). In 65 patients one blood sample was drawn routinely prior to the next morning dose.

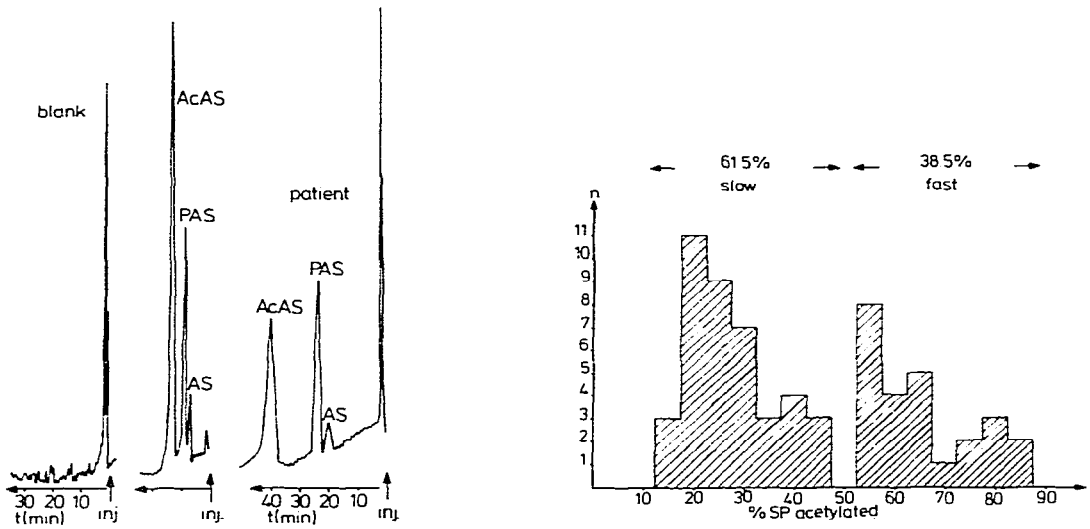

Fig. 2. Typical chromatograms of AS, its acetylated metabolite, AcAS, and the internal standard, PAS. For comparison an extract of a blank plasma (left) and of a patient's sample (right) was injected on to the column. Column: RP-18, 10 μm, 250 × 3 mm. Mobile phase: 50% methanol, 1 g NR_4Br/l. Flow-rate 1.3 ml/min. Excitation: 310 nm. Emission: 430 nm.

Fig. 3. Histogram of the genetically controlled acetylation phenotype of SP in 65 patients with Morbus Crohn or ulcerative colitis.

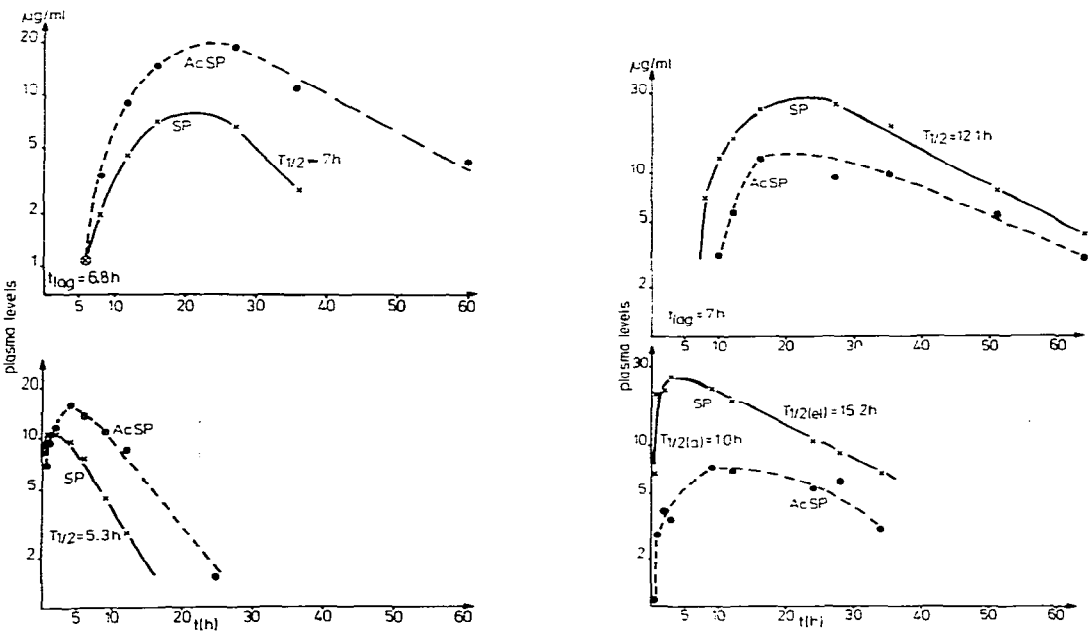

Fig. 4. Plasma level—time profiles: x, SP; •, AcSP. Both in the same fast acetylator following a single oral dose of 4 g SASP (top) and the direct oral application of 1.25 g SP (bottom).

Fig. 5. Plasma level—time profiles: x, SP; •, AsSP. Both in the same slow acetylator following a single oral dose of 4 g SASP (top) and the direct oral application of 1.25 g SP (bottom).

RESULTS

The magnitude of the plasma concentrations of SP and its metabolites are dependent on the genetically determined phenotype. The results of these estimations are given in a histogram, Fig. 3. From the 65 patients tested 40 (61.5%) can be regarded as slow acetylators with an acetylation rate of 12 to 7%.

In Fig. 4 typical plasma level time curves of SP and AcSP following the single oral doses of 4 g SASP (top) and 1.25 g SP in suspension (bottom) in one individual are shown. Following the administration of SASP, SP could be detected with a lag time of 6.8 h. After this time also, the metabolite was visible. Maximal concentrations were reached within 24 h followed by a monoexponential decline with an elimination half-life ($t_{1/2}$) of about 7 h. The time profile of the major metabolite AcSP was similar, but its concentrations were much higher than those of SP in this so-called fast acetylator.

After direct application of SP the drug could be measured already in the first plasma sample (0.5 h). Absorption was rapid with a $t_{1/2}$ between 0.3 and 2 h. Following the peak level the decline of the concentrations could be characterised by a $t_{1/2}$ of 5.3 h. Again, the concentrations of the metabolite exceeded those of the parent compound.

In Fig. 5 the plasma level:time profiles of a slow acetylator were monitored. Again, after oral administration of SASP a lag time and after direct application of SP, rapid absorption was found. While the shape of the curves is similar, significant quantitative differences exist during the elimination phase and in the ratio of the concentrations: $t_{1/2}$ is prolonged to 12—15 h and plasma levels of the metabolite are considerably lower than the corresponding SP values.

In Fig. 6 plasma levels of AS and AcAS are shown in a fast acetylator (top)

TABLE I

MEAN VALUES OF IMPORTANT PHARMACOKINETIC PARAMETERS OF SP IN 7 SLOW ACETYLATORS AND 4 FAST ACETYLATORS.

Parameter	Slow acetylators	Fast acetylators
A. Following the SASP-dose		
Lag time (h)	5.5	
t_{max} (h)	19.8	
$t_{1/2}$ (A) (h)	4.6	
C_{max} (μg/ml)	30.5	7.2
$t_{1/2}$ (el) (h)	15.2	6.6
B. Following the SP-dose		
t_{max} (h)	2.8	
$t_{1/2}$ (A) (h)	1.1	
C_{max} (μg/ml)	21.6	16.7
$t_{1/2}$ (el) (h)	13.0	4.6
Cl (ml/min)	48	155
Vd_{β} (l/kg)	0.8	1.2
Plasma protein binding		
SP = 49.6%	AcSP = 73.5%	

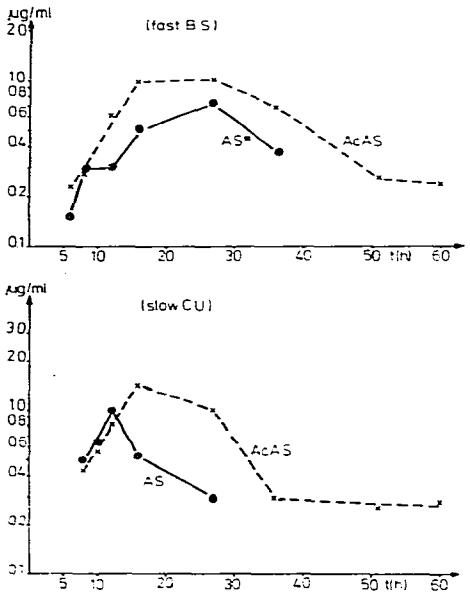

Fig. 6. Plasma level—time profiles: SA and AcAS in a fast acetylator (top) and a slow acetylator (bottom) following the oral administration of 4 g SASP (measured fluorometrically).

and a slow acetylator (bottom) following the single oral dose of 4 g SASP. The low concentrations demonstrate some fluctuations and in all cases the metabolite exhibits higher concentrations than the original compound.

From the decline of the SP concentrations and the area under these curves the most important pharmacokinetic parameters can be calculated. They are summarized in Table I. In the upper part of the table data were obtained after administration of SASP. For more correct calculations SP itself should be administrered as was done in the lower part of this table. According to the concentration ratios the indiviudals were divided into slow and fast acetylators.

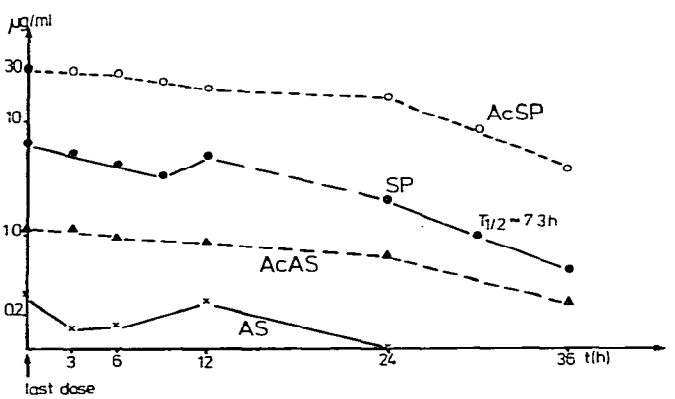

Fig. 7. Plasma level—time profiles: •, SP; x, AS; ○, AcSP; ▲, AcAS. Patient with Morbus Crohn treated chronically with 3 g SASP/die. Treatment stopped for 36 h.

Rate of absorption, either expressed as $t_{1/2}$ (a) or t_{max}, was almost identical in both groups. However, the maximal concentrations of SP were higher in the slow acetylators. A significant difference ($p = 0.0053$) was observed in the elimination $t_{1/2}$. The prolonged $t_{1/2}$ (el) seen in the slow acetylators was due to a much lower total body clearance, $\overline{Cl}$ ($p = 0.0016$). SP and AcSP are bound to plasma constituents to 49.6 and 73.5%, respectively, which corresponds to literature data on the saliva:plasma ratio of 0.5 and 0.25, respectively [8]. The apparent distribution volume, Vd_β, of approximately 1 l/kg was similar in both groups.

In Fig. 7, plasma levels of SP, AcSP, AS and AcAS were monitored for 36 h in a patient treated chronically with 3 g SASP/die. Following the last dose the initial plasma level decline of SP is interrupted by the late absorption peak. Thereafter the concentrations declined with a $t_{1/2}$ of 7.3 h. In this fast acetylator the metabolite exceeds SP. Concentrations of the salicylates are much lower than those of the sulfonamides.

DISCUSSION

The rapid and simultaneous measurements of SP and its major metabolite AcSP in a single plasma sample allows determination of the acetylator phenotype and the plasma levels. Since therapeutic and toxic effects of SP seem to be related to the plasma concentrations, the latter being much more frequent in slow acetylators, these monitorings might improve the safety and effectivity of the therapy with SASP. In addition, knowledge of the pharmacokinetic properties of the administered drugs is an elementary basis for their rational use. It is very important to consider that in the slow acetylators steady state plasma levels of SP will be much higher, since their $\overline{Cl}$-rate is significantly reduced. In the past, SP was regarded as the biologically active moiety of the SASP molecule. However, from two papers published recently [9, 10] it became evident that AS also has a therapeutic effect. So, plasma level measurements of this drug and its major metabolite (AcAS) might be of some help in increasing the therapeutic efficacy of SASP and in evaluating its mechanism of action which might involve the prostaglandins [11].

The high polarity of the salicylates render necessary their extraction into an organic solvent. This problem can be solved by the addition of NR_4Br. The basic NR_4^- ion can form an ion pair with the acidic counter ion (salicylates). AS, AcAS and the internal standard are extracted at pH 7.4 into the organic phase (methylene chloride). The mobile phase (50% methanol) for the elution from reversed-phase HPLC has also to contain NR_4Br, so the compounds are eluted as ion-pairs. The low fluorescence sensitivity of AS does not permit detection of less than a 0.3 µg/ml in a 1-ml plasma sample. However, the HPLC procedure represents a more specific method than the direct fluorescence determination described by Hannson [12].

In applying our methods, 62% of the patients could be regarded as slow acetylators with a $t_{1/2}$ of 13.0 h and 38% as fast acetylators with a $t_{1/2}$ of 4.6 h. This bimodal distribution was caused by significant differences in $\overline{Cl}$.

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