URINARY EXCRETION OF ASPIRIN

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Six human volunteers were each given single oral doses of aspirin (ASA) ranging from 300–1,500 mg. The unchanged ASA excreted in the urine was proportional to dose and urinary pH. The mean percent ($\pm$ s.d.) of dose excreted was 1.9 ± 0.67 . The renal clearance for ASA was 1.42 ± 0.28 l/h. The rate of *in vitro* hydrolysis of ASA to salicylic acid in urine at 37° C was 4 μ g/min for an initial ASA concentration of 7.5 mg in 100 ml human urine.

Introduction

The disposition of acetylsalicylic acid (aspirin, ASA) in man has been reported by a number of workers (Harris & Riegelman, 1967; Rowland & Riegelman, 1967; Rowland *et al.*, 1972; Cham *et al.*, 1980b), and it appears that hydrolysis to salicylic acid (SA) occurs rapidly *in vivo*. Virtually all of a given dose of ASA can be accounted for in terms of SA and its various metabolites (Brooks *et al.*, 1978; Cham *et al.*, 1980a). The recent development of a sensitive and specific h.p.l.c. assay for ASA in plasma (Cham *et al.*, 1980b) has prompted us to apply this assay to urine, and to examine if unchanged ASA appears in urine after oral administration of therapeutic doses of ASA.

Methods

The h.p.l.c. method and preparation of the samples were essentially the same as previously described (Cham, 1980a). The eluent contained 2 ml of butanol, 10 g of sodium sulphate, 5 ml of concentrated acetic acid and 93 ml of water. The flow rate was 3.0 ml/min. The internal standard o-methoxybenzoic acid and ASA were detected at 280 nm.

Various oral doses of soluble effervescent ASA (Aspro-Clear®) ranging from 300 mg to 1500 mg were given to six healthy subjects (aged 23–40 years, weights 50–70 kg). No material was taken to alter urinary pH. Each subject ingested (within 15 s) a given dose of ASA in 250 ml water, prepared just prior to administration. Each study was performed at weekly intervals. Just before ASA ingestion a urine sample was collected and was tested for the presence of ASA or derived metabolites. After ASA ingestion

all urine was collected as frequently as possible at half hourly intervals until no ASA could be detected in the final urine sample. Immediately after collection, urinary pH and volume were measured and ASA concentration was determined.

One ASA tablet (300 mg, Aspro-Clear®) was rapidly dissolved in 100 ml of ASA-free urine. Five ml of this solution was further diluted with 195 ml of ASA-free urine. This solution had a pH of 5.7 and was incubated for 17 h at 37° C. Aliquots were taken at various times for ASA estimation.

Results

The present method revealed that ASA and the internal standard are well separated and the stability of ASA was unaffected by the eluent. Retention times were: ASA 12.9 min; internal standard 15.0 min. No interfering peaks were seen in normal drug free urine samples.

Concentration of ASA and the peak height ratios of ASA to internal standard were linearly related in the concentration range investigated (5.0 to 500.0 mg/l). When 20 μ l sample was injected the limit of accurate measurement was 1 mg/l. The regression equation obtained from data on standards was $y = 0.018x - 0.0008$, $r = 0.9998$, $n = 10$. This equation was not significantly different from 0 in the absence of ASA. Absolute recoveries from urine of ASA at seven concentrations (5.036 to 251.8 mg/l) using four replicates at each concentration ranged from 96.5% to 104%. The degree of *in vitro* hydrolysis of ASA in urine at 37° C was linearly related to time ($r = 0.992$, $P < 0.001$) suggesting a zero-order process. The rate of hydrolysis at pH 5.7 was 4 μ g/min for an initial concentration of 7.5 mg ASA in 100 ml human urine.

Figure 1 shows the relationships of the total

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Figure 1 The relationship of the total amount of acetylsalicylic acid (ASA) excreted and the various ASA doses of six subjects.

amount of ASA excreted unchanged and the various ASA doses of the six subjects. A highly significant correlation is apparent. The linear regression equation of the composite points was $y = 1.392x - 2.219$, $r = 0.9087$, $P < 0.001$. The mean percentage of ASA excretion of all doses (dosage range 300–1500 mg, $n = 27$) was 1.9 ± 0.67 . Figure 2 illustrates the effect of urinary pH on the amount of ASA excreted. A significant correlation was found with a linear equation of $y = 0.7152x - 2.49$; $r = 0.564$, $P < 0.01$.

Regression analysis showed no statistically significant effect of urine volume on the amount of ASA excreted.

Discussion

The method for quantification of SA, salicyluric (SUA) and gentisic (GA) in urine (Cham *et al.*, 1980a) has been successfully extended to measure ASA. In the present study absorbance at 280 nm was monitored for detection of ASA. Dual detection at 280 nm and 313 nm would result in the quantitation of ASA, SA, SUA, GA and in addition may enable the quantitation of the derived glucuronides (Cham *et al.*, 1980a).

To date little attention has been focused on the urinary excretion of ASA. With a single dose of 12 mg/kg it has been shown that the amount of unchanged ASA excreted in urine corresponded to

Figure 2 The effect of the pH in the urine on the amount of acetylsalicylic acid (ASA) excreted.

1.5% of the amount ingested (Cummings & King, 1966). The present study has shown that the absolute amount of ASA excreted is related to the dose. The mean percent of ASA excretion at all doses was $1.9 \pm$

0.67. However, there was a significant correlation between the % of dose excreted and the pH of the urine. Up to 3.2% of a given dose was excreted as unchanged ASA and this occurred within approximately 2 h after dosage. A larger quantity would have been present if continuous urine sampling were possible to overcome the spontaneous hydrolysis of ASA to SA which has been shown to occur at 37° C. No ASA could be detected 3 h after dosage. This observation is concordant with the calculated 13 min elimination half-life of ASA (Cham *et al.*, 1980b).

The total area-under-the-curve ($AUC_{0 \rightarrow \infty}$) for ASA in plasma after a single oral dose of 900 mg soluble effervescent aspirin was $15.32 \pm 5.40 \text{ mg l}^{-1} \text{ h}$ ($n = 5$) (Cham *et al.*, 1980b). In the present study with the same dose and formulation and for the same subjects the cumulative recovery of ASA in the urine was $19.62 \pm 4.34 \text{ mg}$ ($n = 5$). The renal clearance of ASA for the five healthy subjects was $1.42 \pm 0.28 \text{ l/h}$.

The majority of ASA is hydrolysed to SA which, in turn, is eliminated via a variety of rate limited and first order processes (Levy *et al.*, 1972). We have demonstrated that a proportion of the ASA dose, albeit small, is eliminated unchanged. This fraction may be quantitatively more important than GA, which accounts for about 1.3% of a 1.5 g dose of ASA (Cham *et al.*, 1980a) and certainly is more important than gentisuric acid, which has been identified as a metabolite of ASA (Wilson *et al.*, 1978). Thus, pharmacokinetic studies of ASA using urinary data, may overestimate SA clearance if precautions are not taken to inhibit the hydrolysis of ASA to SA.

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References

- BROOKS, P.M., ROBERTS, M.S. & PATEL, B. (1978). Pharmacokinetics of sustained release aspirin. *Br. J. clin. Pharmacol.*, **5**, 337–339.
- CHAM, B.E., BOCHNER, F., IMHOFF, D.M., JOHNS, D. & ROWLAND, M. (1980a). Simultaneous liquid-chromatographic quantitation of salicylic acid, salicylic acid and gentisic acid in urine. *Clin. Chem.*, **26**, 111–114.
- CHAM, B.E., ROSS-LEE, L., BOCHNER, F. & IMHOFF, D.M. (1980b). Measurement and pharmacokinetics of acetylsalicylic acid by a novel high performance liquid chromatographic assay. *Therapeutic Drug Monitoring*, **2**, 365–372.
- CUMMINGS, A.J. & KING, M.L. (1966). Urinary excretion of acetylsalicylic acid in man. *Nature*, **209**, 620–621.
- HARRIS, P.A. & RIEGELMAN, S. (1967). Acetylsalicylic acid hydrolysis in human blood and plasma. Methodology and *in vitro* studies. *J. pharm. Sci.*, **56**, 713–716.
- LEVY, G., TSUCHIYA, T. & AMSEL, L.P. (1972). Limited capacity for salicylphenolic glucuronide formation and its effect on the kinetics of salicylate elimination in man. *Clin. Pharmacol. Ther.*, **13**, 258–268.
- ROWLAND, M. & RIEGELMAN, S. (1967). Determination of acetylsalicylic acid and salicylic acid in plasma. *J. pharm. Sci.*, **56**, 717–720.
- ROWLAND, M., RIEGELMAN, S., HARRIS, P.A. & SHOLKOFF, S.D. (1972). Absorption kinetics of aspirin in man following oral administration of an aqueous solution. *J. pharm. Sci.*, **61**, 379–385.
- WILSON, J.T., HOWELL, R.L., HOLLADAY, M.W., BRITIS, G.M., CHRASTIL, J., WATSON, J.T. & TABER, D.F. (1978). Gentisuric acid: Metabolic formation in animals and identification as a metabolite of aspirin in man. *Clin. Pharmacol. Ther.*, **23**, 635–643.

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