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A study of thiacetazone blood levels and urinary excretion in man, using high performance liquid chromatography

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Summary The pharmacokinetics of thiacetazone, a bacteriostatic drug with both antituberculosis and antileprosy activity, have been studied in healthy volunteers and tuberculosis patients using a high pressure liquid chromatographic method. Urinary excretion of thiacetazone was measured over a period of 7 days following the ingestion of a single oral dose of 150 mg of the drug. Peak plasma concentrations of thiacetazone during supervised daily treatment averaged 1·8 μg/ml. From the rate of decline of thiacetazone plasma concentrations and urinary excretion, it was calculated that thiacetazone concentrations capable of inhibiting the multiplication of *Mycobacterium leprae* would only be maintained for about 3 days in the event of patients discontinuing to take the drug. It was concluded that thiacetazone cannot be recommended for use in the multi-drug treatment of lepromatous leprosy.

Introduction

The clinical activity of thiacetazone (TB1, thioacetazone, *p*-acetylamino-benzaldehyde-thiosemicarbazone) in the treatment of leprosy was first described 30 years ago,[1] while its efficacy when combined with isoniazid for the treatment of pulmonary tuberculosis was demonstrated in a series of controlled clinical trials carried out in East Africa during the 1960s.[2, 3] TB1 continues to be the only commonly available companion drug to isoniazid for use in many Third World countries where the recommended standard treatment for tuberculosis is often 12–18 months' daily isoniazid plus TB1 together with an initial supplement of up to 8 weeks' streptomycin.[3, 4] When TB1 was first used in the treatment of leprosy it was given alone and many patients eventually relapsed, presumably due to the selection of thiacetazone-resistant organisms.[5, 6]

The recent search for drugs of potential use in the multi-drug treatment of lepromatous leprosy which has been stimulated by the increasingly serious

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problem of dapsone resistance, has led to an experimental evaluation of the antileprosy activity of TB1.[7–9] Mouse foot-pad studies have confirmed that TB1 inhibits the multiplication of *Mycobacterium leprae*. Its minimal inhibitory concentration (MIC) was estimated to be about 0·2 μg/ml. However, its activity was primarily bacteriostatic and when TB1 was administered once-weekly its efficacy was substantially impaired.[9] It is known that leprosy patients may ingest their prescribed daily TB1 treatment very irregularly. Thus, in a study carried out in Ethiopia, urine tests demonstrated that only about 30% of the patients had swallowed their TB1 dose within the previous 48 h.[10] Such findings indicate that poor compliance could seriously limit its therapeutic efficacy, particularly if TB1 were rapidly eliminated so that inhibitory levels were not maintained for very long.

Previous estimates of the concentrations of TB1 achieved in the serum after the ingestion of therapeutic doses of the drug (150 mg) were often imprecise because the methods available in the past for its determination were relatively insensitive, non-specific and inaccurate.[11] Thus none of the published ultraviolet or fluorimetric methods[12, 13] were capable of measuring TB1 concentrations below its MIC against *M. leprae* and hence were incapable of directly assessing the duration during which inhibitory concentrations are likely to be maintained after standard dosage. Recently we devised a sensitive and specific high-performance liquid chromatographic (HPLC) method for determining TB1 in plasma and urine and reported preliminary studies on its pharmacokinetics in a single volunteer.[11] This paper describes its application to more extensive studies in a group of healthy volunteers and in tuberculosis patients.

Methods

CHEMICALS

HPLC-grade acetonitrile was purchased from Rathburn Chemicals (Walkerburn, Peebleshire) and other chemicals and solvents were of analytical grade from BDH Chemicals (Poole, Dorset). TB1 was donated by Smith and Nephew (Harlow, Essex) and recrystallized from ethanol. 4-Propylamino-benzaldehyde-thiosemicarbazone (PBT), the propionyl analogue of TB1, was used as the internal standard. Its synthesis has been described elsewhere.[11] Stock solutions (1 mg/ml) of the two thiosemicarbazones were prepared in ethanol and stored at 4°C. Standard curves were prepared by adding appropriate amounts of TB1 and the internal standard to either blank urine or plasma.[11]

PATIENTS AND SAMPLES

The investigation was divided into two parts. Twelve healthy members (2 female,

10 male) of the Medical Research Council's Unit for Laboratory Studies of Tuberculosis and the Royal Postgraduate Medical School Bacteriology Department, aged 19–49 years and weighing 57–95 kg participated in the first study after giving their informed consent. After emptying their bladders, the volunteers swallowed a single capsule containing 150 mg thiacetazone plus 100 mg dapsone plus 6 mg isoniazid[14] some 3 h after a light breakfast and 2 h before lunch. Complete urine collections were then made at 2-hourly intervals up to 6 h and thereafter 1-hour collections from 23·5–24·5, 47·5–48·5, 119·5–120·5, 143·5–144·5 and 167·5–168·5 h. Aliquots of urine were stored at −20°C prior to analysis.

The second study was carried out in 15 tuberculosis patients (3 female, 12 male) from the Infectious Diseases Hospital, Nairobi, Kenya (ages 17–45 years, weights 37–66 kg), undergoing treatment with daily streptomycin (1 g), isoniazid (300 mg) and TB1 (150 mg), their renal and hepatic functions were normal. For the purposes of the study, five consecutive daily doses of the drugs were given under full supervision and food was withheld for 12 h before the last supervised dose to ensure it was taken on an empty stomach. Blood samples (10 ml) were collected immediately prior to the ingestion of this final TB1 dose, then 2, 4, 6 and 24 h afterwards. A light breakfast was taken after the second blood sample. Blood was taken into heparinized tubes and spun down within 1 h of collection. The plasma was then transferred to polypropylene tubes, rapidly frozen and stored at −20°C prior to analysis in London.

ANALYTICAL PROCEDURE

The method employed to extract the samples has been described in detail elsewhere.[11] Briefly, after adding 6 μg of the internal standard (PBT) and 1 M pH 7 phosphate buffer, plasma or appropriately diluted urine samples were extracted with ethyl acetate. Then, after washing with 0·1 M sodium hydroxide, the organic extract was evaporated to dryness under nitrogen. HPLC analyses were performed using a Waters Associates (Northwich, Cheshire) Model M6000A pump, Model 440 ultraviolet detector set at 313 nm and U6K septumless universal injector. A reverse phase system was used consisting of a 25 cm × 5 mm internal diameter 5 μ ODS Hypersil column (Shandon Southern, Runcorn, Cheshire) with a mobile phase of acetonitrile/water (3 : 7 by volume) delivered at a flow-rate of 1·5 ml/min. The minor modifications in the chromatographic method from that published elsewhere[11] were primarily introduced to permit the simultaneous determination of dapsone in the urine samples from the volunteers who received the combined dose (Jenner & Ellard, unpublished results). Neither dapsone, streptomycin, isoniazid or its metabolites acetylisoniazid and isonicotinic acid interfered with the determination of TB1.

Dried urine extracts were dissolved in 100 μl of the mobile phase, duplicate 25-μl aliquots injected and the mean ratio of the peak heights of TB1 to that of the internal standard calculated. Dried plasma residues were extracted by shaking

with 100 μl of the mobile phase together with 100 μl of 2% ethanol in n-hexane in order to remove interfering lipophilic components. After centrifugation, 25-μl aliquots of the lower aqueous phase were injected.

Calibration curves were prepared by spiking blank urine and plasma with TB1 to give concentrations of 0, 0·2, 0·5, 1 and 2 μg/ml, respectively. Duplicate 3-ml aliquots were extracted and chromatographed as described above after the addition of 6 μg PBT. The equations of the lines relating mean peak height ratios of duplicate injections to concentration of TB1 were linear, and the best straight lines and standard errors of slopes and intercepts were calculated by the least squares method.

Results

A representative chromatogram of a plasma extract obtained 2 h after the final supervised dose of 150 mg TB1 from a patient in the East African study is shown in Figure 1. Plasma and urinary concentrations of TB1 were calculated from the peak height ratios of TB1 to PBT and the calibration curves whose equations are given in Table 1 together with the standard errors of the slopes and intercepts. Neither of the intercepts differed significantly from zero. Replicate errors

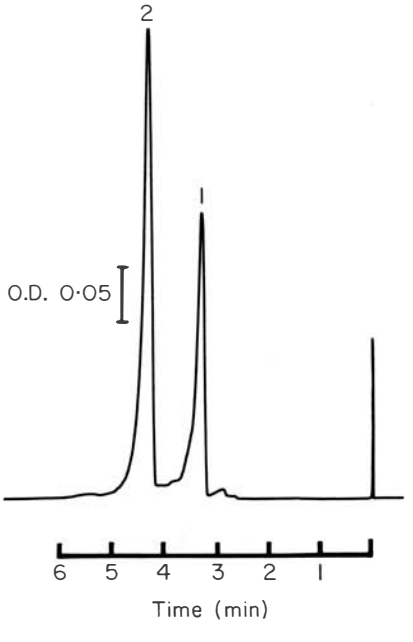

Figure 1. Chromatogram of an extract of plasma from a tuberculosis patient 2 h after the ingestion of the fifth consecutive supervised daily dose of 150 mg TB1 plus 300 mg isoniazid plus 1 g streptomycin. Peaks: (1) TB1; and (2) PBT (the internal standard).

Table 1. Equations* of calibration curves

Biological fluid	Concentration range (μg/ml)	Slope ±s.e.†	Intercept ±s.e.
Urine	0·2–2·0	0·636±0·004	0·012±0·004
Plasma	0·2–2·0	0·636±0·008	0·014±0·009

* Equation $y=mx+c$, where y is the ratio of the peak height of TB1 to that of the internal standard, m is the slope, and x the concentration of TB1.

† Standard error.

calculated from the calibration curves averaged 1·2% for urine and 3% for plasma.

The mean rates of urinary excretion of unchanged TB1 by the 12 healthy volunteers over the 7-day period following the oral ingestion of single 150 mg doses of TB1 are illustrated in Figure 2. Urinary excretion of TB1 diminished more rapidly during the first day (half-life equivalent to 14 h over the period 5–24 h), confirming the biphasic decline encountered in the previous investigation on a single volunteer.[11] From 24 h onwards the apparent half-life for the urinary excretion of TB1 by the 12 volunteers averaged 21·5 h (range 15·8–37·6 h).

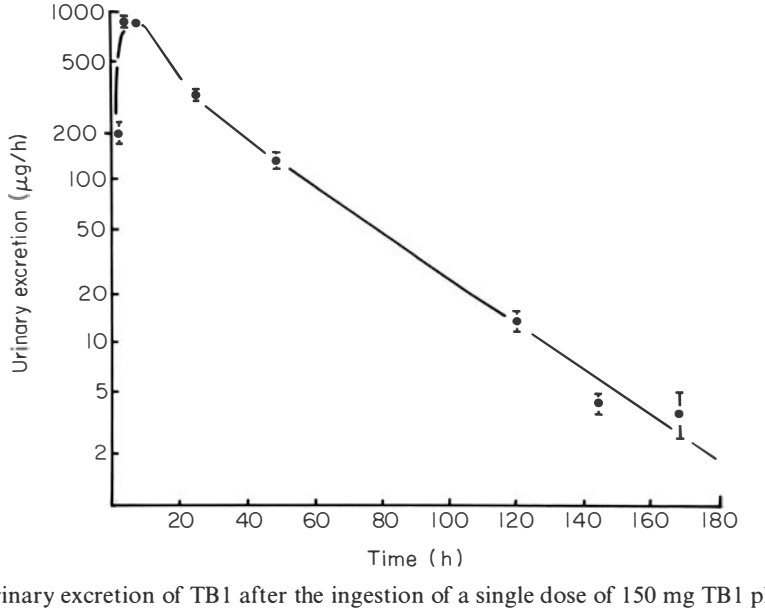

Figure 2. Urinary excretion of TB1 after the ingestion of a single dose of 150 mg TB1 plus 100 mg dapsone plus 6 mg isoniazid. Points represent geometric means and bars show standard errors for the 12 healthy volunteers.

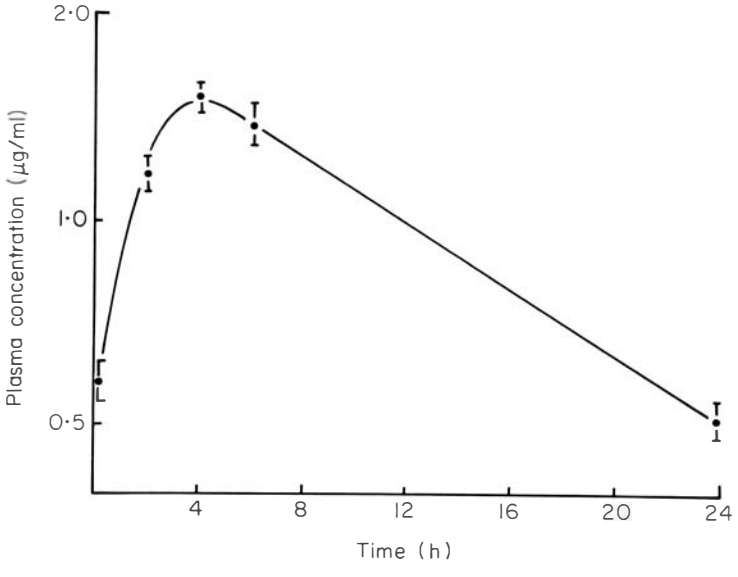

Figure 3. Plasma concentrations of TB1 after the ingestion of the fifth consecutive supervised daily dose of 150 mg TB1 plus 300 mg isoniazid plus 1 g streptomycin. Points represent geometric means and bars show standard errors for the 15 tuberculosis patients.

However, only one of the volunteers had a TB1 half-life over this period in excess of 28 h, a value significantly longer ($P < 0{\cdot}001$) than that of 11 others whose TB1 half-lives did not differ significantly ($P = 0{\cdot}2$).

The mean plasma TB1 concentrations of the 15 tuberculosis patients following the ingestion of the last of the five consecutively supervised daily doses of 150 mg TB1 are illustrated in Figure 3. Peak TB1 concentrations averaged 1·76 μg/ml and were attained within 4 h in all but three of the subjects. From 6 to 24 h TB1 plasma concentrations declined at a rate equivalent to a half-life of 12·9 h. Plasma TB1 concentrations at 0 and 24 h did not differ significantly ($P > 0{\cdot}25$). They averaged 0·65 (SD 0·32) and 0·55 (SD 0·26) μg/ml, respectively. Although an analysis of variance showed that there were significant interindividual differences between patients in their trough TB1 plasma concentrations ($P < 0{\cdot}005$), such differences could not be accounted for by differences in age or weight.

Discussion

A recently devised specific and sensitive HPLC method[11] has been applied to determine the plasma concentrations of TB1 in tuberculosis patients and rates of its urinary elimination in healthy volunteers. This has provided accurate information to assess the potential of the drug when employed as a component of

multi-drug regimens for the treatment of lepromatous leprosy. Peak and trough plasma concentrations of TB1 after daily dosage with 150 mg of the drug averaged 1·76 and 0·60 μg/ml respectively. These mean values are similar to those obtained in an earlier investigation carried out on a large number of tuberculosis patients from Kenya and Singapore using less sensitive and specific ultraviolet and fluorimetric methods.[13] The peak and trough concentrations of TB1 only exceeded its MIC against *M. leprae* by about 9- and 3-fold respectively. When given at a daily dosage of 150 mg, serum concentrations of thiacetazone were unaffected by the co-administration of 1 g streptomycin,[13] while the rate of its urinary excretion was uninfluenced by giving 100 mg dapsone concomitantly (Jenner and Ellard, unpublished results).

From the decline in the rates of TB1 urine excretion beyond 24 h, it may be calculated that inhibitory levels of the drug would only be maintained for about 3 days following cessation of regular daily treatment, a conclusion supported by the study carried out on a single healthy volunteer.[11] This study therefore confirms the previous conclusion[10] that poor compliance would seriously impair the therapeutic efficacy of this inherently weak bacteriostatic drug and that as a consequence TB1 cannot be recommended for general out-patient use in the multi-drug treatment of lepromatous leprosy.[15, 16]

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