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Note

High-performance liquid chromatographic determination of teicoplanin in plasma: comparison with a microbiological assay

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Teicoplanin (Fig. 1) is a novel amphoteric glycopeptide antibiotic that is active in vitro and in vivo against a broad range of Gram-positive bacteria [1,2] It is chemically closely related to the ristocetin–vancomycin group, produced by *Actinoplanes teichomyceticus* [3], and is used for the treatment of human infectious diseases (e g , endocarditis) caused by staphyllococci, streptococci and enterococci, especially if patients are allergic to both penicillins and cephalosporins or when resistance occurs to other antibiotics [4] Teicoplanin posesses a wide therapeutic range without signs of nephrotoxicity or ototoxicity [5].

Our own data [6] show that the clinical efficacy, especially in severe infections, e g , septicaemia, is dose-dependent In these cases proper dosage (infusion) and control of substance levels in body fluids at the beginning of treatment should be performed in order to optimize the clinical efficacy

Several different analytical methods have been used to determine teicoplanin in blood, including agar diffusion tests [7,8], a disc susceptibility test [9] and high-performance liquid chromatography (HPLC) [10] In this paper we report a simple HPLC method for the teicoplanin complex employing Bond Elut (SAX) solid-phase extraction of plasma samples and isocratic reversed-phase (C_8) separation

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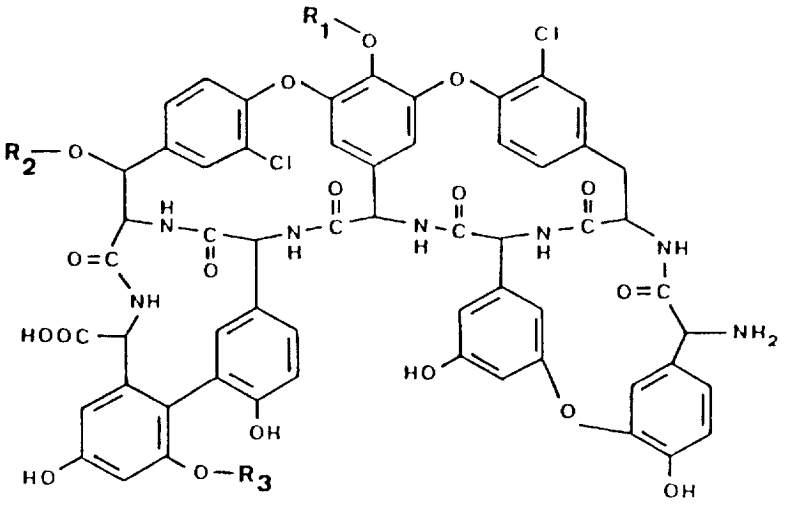

Fig 1 Structure of the major glypopetides of the teicoplanin complex R_1 = amino sugar, R_2 = amino sugar, R_3 = D-mannose

EXPERIMENTAL

Chemicals

Teicoplanin was supplied by Merrell Dow (Russelsheim, F R G) and disodium *n*-heptanesulphonate (Sigma, Heidelberg, F R G) served as an ion-pair-forming agent. For the mobile phase, deionized, distilled water and HPLC-grade methanol (J.T. Baker, Deventer, The Netherlands) was used. Sodium acetate and glacial acetic acid (both Merck, Darmstadt, F R G) were used for aqueous buffers (pH 4 0) The HPLC solvent was degassed and filtered prior to use through a 0 45-μm FUHP filter from Millipore (Bedford, MA, U S A) The sample clean-up cartridges employed were SAX Bond Elut columns from Analytichem (Harbor City, CA, U S A.)

HPLC equipment

The liquid chromatograph was from Kontron (Vienna, Austria), consisting of a Model 420 pump, a Model 830 column oven, a Model 460 intelligent autosampler and a Uvikon 430 photometric detector A LiChrosorb RP-8 (10 μm) guard column (10 mm$\times$4.2 mm I D) and a LiChrosorb RP-8 (5 μm) analytical column (150 mm$\times$4.2 mm I.D.) were used Columns were connected by an Eco-tube cartridge system (Bischoff, Leonberg, F.R.G.)

Chromatographic conditions

The mobile phase was methanol–water (5 95, v/v) containing 0 01 mol/l disodium *n*-heptanesulphonate and was adjusted to pH 4 0 with sodium acetate

(2 g/l) and glacial acetic acid. The HPLC system was operated at 0 8 ml/min (pressure 59 bar) and thermostated at 30°C. Detection was performed by measuring the absorbance at 240 nm (sensitivity 0 005 a u f s with a response time of 2 s)

Sample handling

Teicoplanin was administered via bolus injection into the cubital vein (400 mg in 5 ml of sterile sodium chloride solution) Blood samples were obtained by venepuncture of teicoplanin-treated patients 30 min and 24 h after administration, collected in heparinized tubes, centrifuged and the supernatant was stored at −70°C until analysis

Extraction procedure

SAX Bond Elut sample clean-up cartridges were placed on the Bond Elut extraction unit and prepared prior to use by washing with 4 ml of methanol, 1 ml of distilled water and 1 ml of *n*-heptanesulphonic acid (0 01 mol/l) A 1 0-ml volume of plasma was diluted with 1.0 ml of *n*-heptanesulphonic acid (0 01 mol/l), vortex-mixed, centrifuged at 4000 *g* for 2 min and forced through the extraction cartridges by vacuum. The plasma constituents were washed out from the cartridge with 3 0 ml of *n*-heptanesulphonic acid (0 01 mol/l) and teicoplanin finally was eluted with 1.0 ml of methanol An aliquot of 20 μl was injected on to the column

RESULTS AND DISCUSSION

Microbiological assays are widely used to effect quantitation of the drug Teicoplanin is a mixture of at least six major components, which have been separated recently by affinity and reversed-phase chromatography [11] The main components were determined by gradient elution with high recovery and precision of the extraction step However, quantitation was performed by comparing the sums of the areas under the six major peaks, which was necessary because each component has its specific antibacterial activity In our studies, there is no need to separate the major teicoplanin components and therefore we tried to analyse the sum of the compounds as a single peak in order to reduce the time required for the assay

Extraction

The extraction procedure is similar to that for vancomycin assay reported by Greene et al. [10] The recovery for samples in the concentration range 2–60 μm/ml was 90–95% with a relative standard deviation of 7%

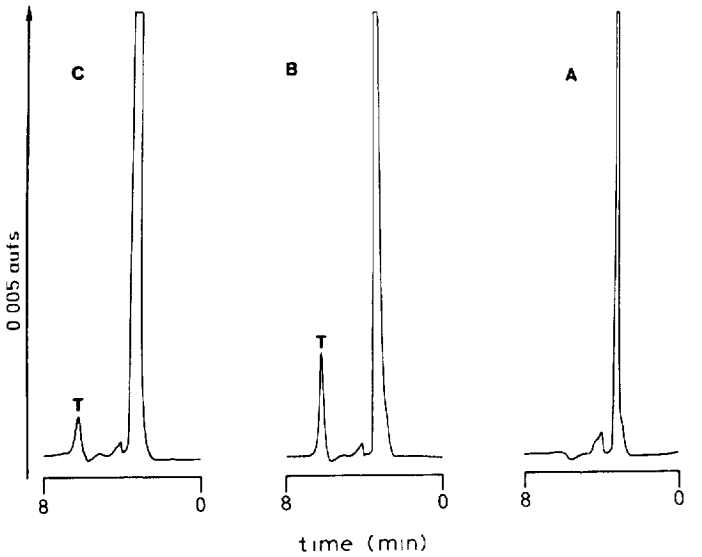

Fig 2 Chromatograms obtained from extracts of (A) blank plasma and plasma samples spiked with (B) 10 μg/ml and (C) 3 μg/ml teicoplanin (T)

Chromatography

The retention time of the teicoplanin peak was 6 5 min (Fig 2) The complex of teicoplanin is well separated and there is no overlap with the matrix peaks in front of the chromatogram Analysis is performed within 10 min

Quantitation

Quantitation was effected by the external standard method Standard samples containing 5, 10, 20 and 40 μg/ml were prepared by dilution of a fresh stock solution with 0 01 mol/l *n*-heptanesulphonic acid Standard samples were extracted in the same way as plasma samples In a series of thirty samples a three-point calibration graph was run after each ten samples Concentration was calculated comparing peak heights of samples with the calibration graph, which was linear in the range 3–50 μg/ml and can be expressed by the equation $y=0\ 62x-\ 1\ 81$ ($R=0\ 997$, $n=6$), where y is teicoplanin concentration and x is the peak height (mm) The detection limit was in the range 0 2–0 4 μg/ml on injecting 20 μl of sample extract This limit is sufficient because of the high plasma levels of the drug

Microbiological assay (MA)

Serum teicoplanin concentrations were determined by an agar diffusion method using a test strain of *Bacillus subtilis* For serum analysis, three different dilutions of samples were pipetted into agar plates in triplicate and the

emerging inhibition areola was read instrumentally The calibration graph was obtained in a similar way All samples were incubated overnight for 18 h The detection limit of our MA is in the same ranges as that of the HPLC assay (0 2 μg/ml)

Comparison of HPLC with microbiological assay

Fig 3 depicts the correlation of results in the MA and HPLC assay of teicoplanin There is a linear correlation between both methods over the whole serum concentration range. The fitted curve can be expressed by the equation $y=0\,93x+0\,885$ ($n=30$, $r=0\,940$, $p\leqslant 0\,0001$), where x represent the HPLC values and y the MA values

Table I summarizes the advantages and disadvantages of both methods Despite the expense HPLC analysis is preferred in our laboratories owing to the low time consumption and the rapid results The HPLC method displays a good reproducibility with a standard deviation of 3–4% ($p=0\,01$) The standard deviation for our MA assay is in the range 5–7% ($p=0\,01$)

Although MA may not be specific because bacteriologically active metabolites or degradation products might interfere with the determination of the drug, this was the only method available to date Our MA requires substantial time for preparation and evaluation The incubation time is approximately 18 h and data are available within one day [8,12,13] The evaluation of the assay

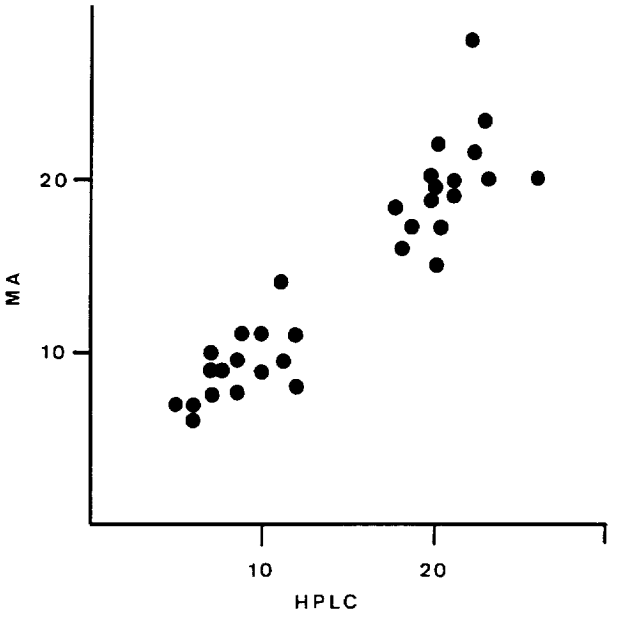

Fig 3 Plasma concentrations of teicoplanin obtained by microbiological assay (MA) and liquid chromatography (HPLC) (data in μg/ml)

TABLE I

COMPARISON OF TEICOPLANIN ASSAY BY AGAR DIFFUSION TEST WITH COLUMN LIQUID CHROMATOGRAPHY

Parameter	MA	HPLC
Time for sample preparation, including agitation, centrifugation and cartridge preparation (HPLC)[a]	1 min	2 min[b]
Time to obtain results[a]	26 h	0 5 h
Limit of detection	0 25 μg/ml	0 2 μg/ml
Costs[a]	Low	High
Costs for co-workers	High	Low
Automation	Not possible	Easy
Presentation of results	Difficult	Easy

[a]Required for one sample
[b]Mean time for one sample if at least five samples are treated simultaneously

may be difficult in cases of unsymmetrical diffusion or vague borderlines The standard error for the microbial assay is greater than for HPLC working with an external standard On the other hand, the concentration of all active metabolites is determined in one step However, in drug monitoring in a clinic, there is a need for rapid availability of results in order to be able to optimize the dosage The HPLC method offers a rapid determination of teicoplanin in patients' serum The results of analysis are available 30 min after collection of a plasma sample (including extraction, chromatography of the sample and standard and calculations) Therefore, doubtful results can be rapidly checked by a new analysis With the exception of sample preparation, all steps in the analysis and data evaluation can be fully automated To obtain a lower standard deviation, vancomycin can be used as an internal standard However, a fully automated HPLC assay costs approximately four times as much as an MA

The MA method is to be preferred for experimental research with a large number of samples to be evaluated owing to the low costs Also, in laboratories with little experience of HPLC or sample preparation there should be consideration as to whether to use HPLC or not On the other hand, when rapid analytical results are required, HPLC is the method of choice Our first results have encouraged us to use HPLC to an increasing extent for monitoring teicoplanin in patients at the start of treatment

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