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Determination of telithromycin in human plasma and microdialysates by high-performance liquid chromatography

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Abstract

A high-performance liquid chromatography method for the quantitative determination of telithromycin in biological fluids is described. The method is suitable for plasma and microdialysates from the interstitial space fluid of skeletal muscle and subcutaneous adipose tissue. Plasma samples were deproteinised with trichloroacetic acid and neutralised with sodium hydroxide. Microdialysates were analysed without further preparation step. Telithromycin was separated isocratically on a reverse-phase column using acetonitrile–0.03 M ammonium acetate, pH 5.2 (43:57, v/v) at a flow rate of 0.8 ml min^{-1}, and fluorescence detection (excitation 263 nm, emission 460 nm). The calibration curve was linear from 0.01 to 5 μg ml^{-1}. Within- and between-day imprecision and inaccuracy was ≤10%. The limits of quantification were 0.02 and 0.015 μg ml^{-1} for plasma and microdialysates, respectively. Since telithromycin is decomposed in aqueous solution at ambient temperature, it is strongly recommended to store samples frozen at −80 °C, to maintain the temperature at 4 °C during all preparation steps, and to analyse samples within 120 min after thawing.

Keywords: Telithromycin; Ketolide; Microdialysis; High-performance liquid chromatography

1. Introduction

Ketolides, a novel family of semi-synthetic antimicrobial agents, were designed to overcome the increasing resistance of gram-positive cocci against macrolides [1]. The first member of ketolides introduced into clinical practice is telithromycin, a compound which was derived from the macrolide erythromycin-A by substitution of a keto group for L-cladinose at the three-position and addition of an N-substituted 11,12-carbamate side chain [1]. Although primarily developed for the treatment of respiratory tract infections, telithromycin has been also speculated to be an option for the therapy of skin and soft tissue infections due to its extended in vitro antibacterial spectrum [2]. Since the outcome of antiinfective treatment depends on the pharmacokinetic profile of the used antimicrobial agent at the target site [3], a clinical study was carried out to determine the concentration of telithromycin in the interstitial space fluid of soft tissues after oral administration of a single dose to young healthy volunteers [4]. In this study, the microdialysis (MD) technique, a well established in vivo method for the continuous monitoring of free drug concentrations in the interstitial fluid of various tissues [5,6], was applied. MD has been employed in a number of other pharmacokinetic studies in patients and healthy volunteers [4,7–10]. Samples obtained by MD require sensitive detection, because the sample volumes are small, i.e. typically around 30 μl, and the analyte concentrations at the latest time points of sampling can be in the nanomole range [4,5]. The concentration depends on

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the pharmacokinetic properties of the drug and the diffusion capacity of the microdialysis membrane for the respective drug.

In the present study we describe a reversed-phase HPLC method for the quantitative assessment of telithromycin in plasma and dialysates derived by MD. In contrast to macrolides, the imidazolyl and pyridyl ring attached to the carbamate side chain of telithromycin allows fluorescence detection which ensures sufficient sensitivity of the assay. There are, however, some details one should comply with concerning the stability of telithromycin in aqueous solution at room temperature.

2. Experimental

2.1. Reagents and standard solutions

Pure telithromycin was a gift from Aventis Pharma (Frankfurt, Germany). Ringer's solution was purchased from Mayrhofer Pharmazeutica GmbH (Linz, Austria). All other solvents and reagents were from Sigma–Aldrich (Steinheim, Germany).

Stock solutions of telithromycin were prepared by dissolving 10 mg in 10 ml acetonitrile and stored at −20 °C. Calibration standards for the microdialysates were prepared on ice by further diluting the stock solution with Ringer's solution. Calibration standards for the plasma samples were prepared by spiking plasma with telithromycin at concentrations ranging from 0.01 to 5 μg ml^{-1}. Aliquots of all calibration standards were stored immediately at −80 °C.

2.2. Sample preparation

All samples were stored at −80 °C until analysis. Plasma samples were placed on ice immediately after thawing for 20 min at ambient temperature. For all further preparation steps samples were kept at 4 °C. An aliquot of 540 μl plasma was deproteinised by vortex-mixing with 60 μl ice-cold 50% trichloroacetic acid and centrifugation at 12,000 × *g* for 10 min. 10 μl 2.5 M sodium hydroxide was added to 200 μl of the supernatant to achieve a pH of 5.

As microdialysates are free from proteins, they can be analysed without further preparation. For all samples, the time between thawing and analysis was lower than 120 min. The volume of injection was 15 μl.

2.3. Chromatographic conditions

The HPLC system consisted of a Beckman System Gold 126 pump connected to a 508 autosampler (Beckman Coulter Inc., Fullerton, CA, USA), a Jasco FP-920 fluorescence detector (Jasco, Tokyo, Japan) and a PC using Beckman 32 Karat integration software. Isocratic separation was carried out at ambient temperature with a LiChroCART RP-18e 250 mm × 4.0 mm, 5 μm particle size column (Merck, Darmstadt, Germany) which was protected by a guard column (LiChroCART RP-18e 4 mm × 4 mm; Merck, Darmstadt, Germany). The mobile phase was composed of a solution of ammonium acetate (0.03 M), adjusted to pH 5.2 with acetic acid, and acetonitrile in a 59:41 volume ratio. The flow rate was 0.8 ml min^{-1}. The spectrofluorometer was set at 263 and 460 nm for the excitation and emission wavelength, respectively.

2.4. Quantification and validation

Ten-point calibration curves were constructed from the peak area vs. concentration data of the standards in the range 0.01–5 μg ml^{-1}. Recoveries of telithromycin are expressed as the ratio of peak areas of spiked and processed plasma samples at concentration 0.05, 0.5 and 1 μg ml^{-1} and corresponding peak areas from directly injected standards in Ringer's solution in percent. The method was validated by analysis of standards at five different concentrations (0.015, 0.25, 0.5 and 1 μg ml^{-1}) in six replicates on four different days. Imprecision was given as the relative standard deviation (R.S.D.). Inaccuracy was determined as follows: Inaccuracy % (bias) = ((observed mean concentration − nominal concentration)/nominal concentration) × 100. The limit of quantification was defined as the lowest concentration at which the R.S.D. is <15%. The limit of detection was defined as the lowest concentration with a signal-to-noise ratio of 3. Stability data were obtained by monitoring peak areas of standard solutions (0.1 and 1 μg ml^{-1}) over a period of 48 h at room temperature and at 4 °C.

2.5. Protein binding study

Aliquots (0.3 ml) of plasma were ultrafiltrated by centrifugation at 12,000 × *g* for 60 min at room temperature by using centrifugal filter units equipped with a low-binding regenerated cellulose membrane (nominal molecular mass limit 5000; Ultrafree-MC; Millipore Corp., Bedford, Mass.). For determination of the binding of telithromycin to the membrane, standards of telithromycin diluted in Ringer's solution (0.5, 1, 2.5 and 10 μg ml^{-1}) were ultrafiltrated in the same way. Ultrafiltrates were analysed by using spiked standards for calibration. Concentrations obtained from plasma ultrafiltrates were corrected with the membrane binding ($C_{\text{corr UF plasma}}$). Protein binding (%) was calculated using the equation $100 - (C_{\text{corr UF plasma}}/C_{\text{plasma}}) \times 100$.

3. Results and discussion

3.1. Stability and recovery

Telithromycin was found to be unstable in aqueous solution at ambient temperature. The conformational change, possibly the hydrolysis of the ester bond of the carbamate side chain, resulted in reduction of the measured concentration

with a half-life of 21.2 and 23.5 h for standards in Ringer's solution and processed plasma samples, respectively. When samples were strictly kept at 4 °C the half-life was prolonged to approximately 42 and >48 h, respectively. Samples frozen at −80 °C were found to be stable for at least 3 months. It is recommended to thaw the samples in sets of 12 vials at most, prepare them on ice and cool the autosampler tray. The concentration changes in adequately processed samples are within the inaccuracy of the assay if analysed within 120 min after thawing. The recovery of telithromycin from plasma ranged from 93.3 to 101.2%.

3.2. Sample preparation and chromatography

The described preparation steps for plasma samples result in clear supernatants of the same pH as the mobile phase. Chromatograms of blank plasma from male healthy volunteers showed no interfering peaks in the region of interest. Telithromycin was eluted as a sharp peak with a retention time of 6.42 ± 0.06 min and a peak symmetry better than 1.5 (measured at 5% peak height). Typical chromatograms are shown in Fig. 1. With replacement of the guard column after every 300–400 injections, more than 1200 analyses could be performed on the same analytical column without loss of peak quality. Similar chromatographic conditions are briefly described in the method section of a study reporting the pharmacokinetics of telithromycin and related compounds in guinea pigs [11]. In this method, plasma samples were evaporated to dryness after deproteinisation, which is time-consuming and bears the risk of decreasing telithromycin concentrations due to hydrolysis.

3.3. Validation

Since telithromycin to date is the only ketolide on the market, a compound with similar structure as internal standard was not available. The sample preparation without extraction procedures allows quantification with plain external calibration. Validation coefficients were ≤10% and thus met the acceptance criteria specified within the guidelines for the validation of analytical methods [12]. Values of within- and between-day imprecision and inaccuracy are summarized in Tables 1 and 2.

Table 1
Within-day imprecision (R.S.D.%) and inaccuracy (bias%)

Telithromycin concentration (μg ml^{-1})			
Nominal	Measured (mean)	R.S.D. (%)	Bias (%)
Plasma (n = 6)			
1.000	0.993	2.2	−0.7
0.500	0.487	2.8	−2.6
0.250	0.246	7.3	−1.6
0.050	0.046	7.7	−8.0
0.020	0.021	9.2	+5.0
Ringer's solution (n = 6)			
1.000	0.989	1.6	−1.1
0.500	0.491	2.4	−1.8
0.250	0.244	6.1	−2.4
0.050	0.244	6.1	−2.4
0.015	0.016	8.8	+6.6

Table 2
Between-day imprecision (R.S.D.%) and inaccuracy (bias%)

Telithromycin concentration (μg ml^{-1})			
Nominal	Measured (mean)	R.S.D. (%)	Bias (%)
Plasma (n = 24)			
1.000	0.987	3.1	−1.3
0.500	0.491	3.1	−1.8
0.250	0.244	6.4	−2.4
0.050	0.048	9.3	−4.0
0.020	0.018	9.5	−10.0
Ringer's solution (n = 24)			
1.000	1.012	4.3	+1.2
0.500	0.509	2.7	+1.8
0.250	0.241	6.1	−3.6
0.050	0.046	8.2	−8.0
0.015	0.016	7.9	+6.6

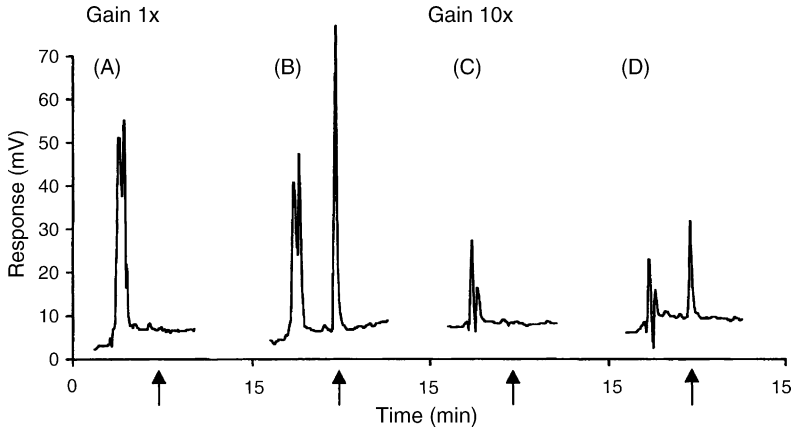

Fig. 1. Chromatograms of plasma and microdialysate. (A) Drug-free plasma; (B) plasma of a volunteer 140 min after oral administration of 800 mg telithromycin (1.2 μg ml^{-1}); (C) drug-free microdialysate; (D) microdialysate from interstitial space fluid of subcutaneous adipose 260 min after oral administration of 800 mg of telithromycin (0.038 μg ml^{-1}). Arrows indicate the retention time of telithromycin (6.42 ± 0.06 min). Data were corrected for the individual microdialysis recovery values.

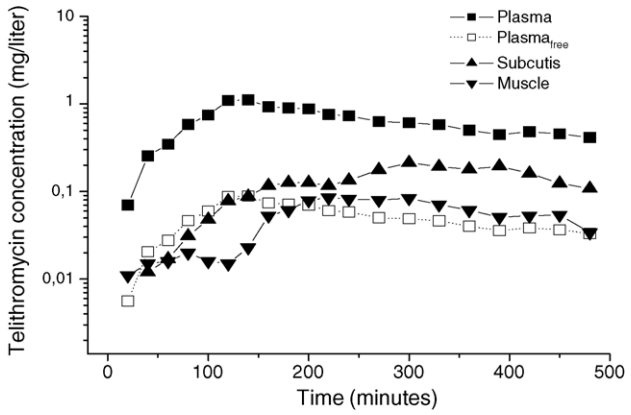

Fig. 2. Time vs. concentration profile of total and free telithromycin in plasma, skeletal muscle, and subcutis in a healthy male volunteer after oral administration of 800 mg. Plasma concentrations of free telithromycin were calculated from the individual plasma protein binding. Data were corrected for the individual microdialysis recovery values.

3.4. Linearity and limit of quantification

The calibration curve was linear over 0.01–5 $\mu g\,ml^{-1}$ for both matrices ($R > 0.999$). The limit of quantification was 0.02 and 0.015 $\mu g\,ml^{-1}$ in plasma and Ringer's solution, respectively. The limit of detection was 0.005 for plasma and 0.002 $\mu g\,ml^{-1}$ for Ringer's solution.

3.5. Pharmacokinetics of telithromycin

Results of the pharmacokinetic study in which the assay has been applied are publicated elsewhere [4]. Fig. 2 shows a representative time versus concentration profile in plasma, skeletal muscle and subcutis of a male healthy volunteer after oral administration of a single dose of 800 mg of telithromycin. The mean plasma protein binding of telithromycin in 10 volunteers was $88.5 \pm 1.0\%$ which is in accordance with findings in the literature [13].

3.6. Conclusion

The described method for the measurement of telithromycin in human plasma and microdialysates can be performed with basic instrumentation of the chromatographic laboratory. The relative instability of the compound, however, does not allow automated over-night runs of large numbers of samples, as usually performed in the analytical routine.

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