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Quantitative determination of individual teicoplanin components in human plasma and cerebrospinal fluid by high-performance liquid chromatography with electrochemical detection

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Abstract

We have developed a simple, rapid and highly sensitive method for determining a plasma or cerebrospinal fluid (CSF) concentrations of individual teicoplanin components using reversed-phase high-performance liquid chromatography followed by electrochemical detection. A linear relationship was observed between concentrations and peak heights for the teicoplanin concentration range of 0.025–10 μg/mL. The correlation coefficients of all standard curves were greater than or equal to 0.999. The limit of detection for the major component of teicoplanin was 1.0 ng/mL (signal/noise ratio >3). Daily fluctuations of standard curves ($n = 5$) were small, with coefficients of variation of 3.3%. The intra-assay precision was 5.9% ($n = 5$). Inter-assay precision ranged from 2.6 to 6.8%. The method described here is suitable for clinical monitoring of teicoplanin levels in plasma or CSF level and for use in studies involving pharmacokinetics of individual teicoplanin component.

Keywords: Teicoplanin; Cerebrospinal fluid; HPLC; Electrochemical detection

1. Introduction

Teicoplanin is effective against most Gram-positive aerobic and anaerobic organisms [1]. Teicoplanin is a group of glycopeptides consisting of six major closely related molecular species and other minor components [2]. The antimicrobial activities of these components against some microbial species are different [3]. Fluorescence polarization immunoassay, which is a widely used and highly convenient method for determining the plasma concentration of teicoplanin, does not allow separate determinations of individual components of teicoplanin. Some high-performance liquid chromatographic (HPLC) methods for the analysis of teicoplanin components in human plasma have been reported [4–10]. These HPLC methods are, however, limited by one or more factors, such as detection limitation, potential interference from co-eluting matrix constituents and time-consuming extraction procedures. Furthermore, to date, there are no published data on the teicoplanin concentration in cerebrospinal fluid (CSF). Because of detection limitation, the HPLC methods reported so far cannot be used to determine teicoplanin concentrations in CSF. Monitoring of teicoplanin in CSF may be helpful for certain patient groups to ensure that therapeutic concentrations are maintained.

In this study, we developed a simple, rapid and highly sensitive method for determining plasma and CSF concentrations of individual teicoplanin components using reverse-phase HPLC followed by electrochemical detection.

2. Experimental

2.1. Materials and sample preparation

Teicoplanin was provided by Astellas Pharma Inc. (Osaka, Japan). Methanol and acetonitrile were purchased from Wako

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Pure Chemical Ind. Ltd. (Osaka, Japan). All other reagents were of the highest grade available commercially and were used without further purification.

The study was performed in the Intensive Care Unit (ICU) of the Department of Medicine at Hokkaido University Hospital. A 9-year-old female living donor liver transplant recipient who had primary biliary cirrhosis was enrolled in this study. The Ethics Committee of Hokkaido University Hospital approved this study, and written consent was obtained. Teicoplanin was administered as treatment for meningitis. After administering an intravenous bolus of 100 mg of teicoplanin, blood samples were collected at given times up 24 h and CSF sample was collected 24 h after administration for measurement of teicoplanin concentrations.

Extraction of teicoplanin from plasma or CSF samples was achieved by a filtration to remove interfering compounds. Plasma or CSF sample (100 μL) was loaded onto a Millex-HV cartridge (Millipore, MA, USA) to remove interfering compounds. After centrifugation (3000 × *g*, 5 min, room temperature), the 10 μL of the filtrate was used for HPLC analysis. The adsorption of teicoplanin onto the filtration membrane was negligible. Water used as the mobile phase in the extraction procedures was purified by Millipore Milli-Q water filtration system (Millipore, MA, USA).

2.2. Calibration standards

A stock solution of teicoplanin (40 μg/mL) was prepared by dissolving appropriate amounts in distilled water. The stock standards of teicoplanin were diluted with water, giving working solutions ranging from 0.025 to 10 μg/mL. Stock and working solutions of teicoplanin were stable for at least 1 month without observable degradation when stored at −20 °C.

2.3. HPLC Analytical methods

Teicoplanin concentrations were determined as follows: an HPLC system (LC-10AD pump, Shimadzu, Kyoto Japan) equipped with a CoulochemII electrochemical detector, an analytical cell (No. 5012) and a guard cell (No. 5020) (ESA, MA, USA) was used with a reversed-phase column (CAPCELL PAK C8, 150 mm × 4.6 mm i.d., particle size: 5 μm) (Shiseido, Tokyo, Japan). The assay was performed at room temperature. The mobile phase consisted of acetonitrile and 100 mM phosphate buffer (pH 4.4) (15:85, v/v) and was used at a constant flow rate of 0.6 mL/min. The detector cell potential for oxidation was examined from +300 to +1000 mV to obtain current–voltage relationships.

2.4. Cyclic voltammetry

Cyclic voltammetry (CV) of teicoplanin was developed by injecting 10 μL of 10 μg/mL concentration of standard solution. The current was recorded over a voltage range of +200 to +1000 mV. At the end of analysis, the current generated was plotted against the potential applied.

2.5. Data analysis

The components of each eluted peak were identified by the results of the monoisotopic mass values and compared to the report by Ackermann et al. [6]. The molar concentration of teicoplanin components was calculated based on their molecular weights. The relative ratios of the heights of individual components on HPLC chromatogram of the authentic sample were assumed to correspond to their molar ratios.

3. Results and discussion

3.1. Setting condition for teicoplanin analysis

The current–voltage relationship (current produced versus potential applied) is shown in Fig. 1. Higher peak current was obtained at higher applied potential, though a longer time was required to stabilize the HPLC system. The E_2 potential was set at +800 mV for suitable determination of teicoplanin. The potential of guard cell was set at +850 mV and potential of E_1 cell was set at +300 mV because teicoplanin is hardly oxidized. The best separation was observed by using phosphate buffer at pH 4.4, in which a higher current is generated (Fig. 2) without interfering with background peaks.

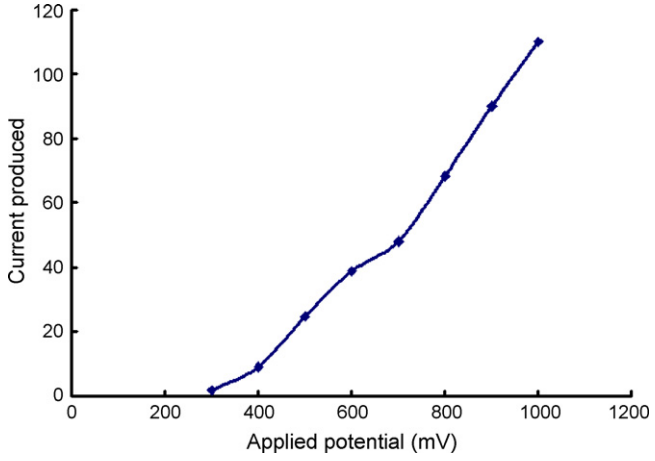

Fig. 1. Cyclic voltammetry (CV) of teicoplanin. The CV was developed by plotting the current (nA) produced by 10 μL of teicoplanin (10 μg/mL) against various potentials applied.

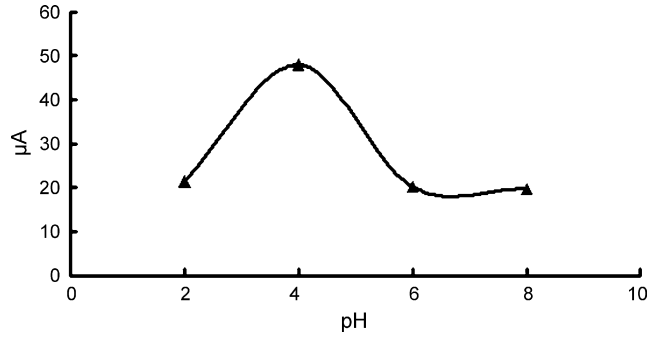

Fig. 2. Effect of pH of mobile phase on current generated. The pH of 100 mM phosphate buffer has been changed.

Calibration curves were determined by least-squares linear regression analysis. A linear relationship was obtained between concentration and peak height of each component for the teicoplanin concentration range of 0.025–10 μg/mL in plasma or CSF. The correlation coefficients of all standard curves were greater than or equal to 0.999. The limit of detection for plasma or CSF of each teicoplanin component was 1.0 ng/mL (signal/noise ratio >3). The daily fluctuation in CSF standard curves ($n = 5$) was small, with a coefficient of variation (CV) of 3.3%. The intra-assay precision for the teicoplanin samples was 5.9% ($n = 5$). Inter-assay precision was evaluated by comparing the data obtained on three validation days, and the results ($n = 3$) ranged from 2.6 to 6.8%. Recoveries of teicoplanin from spiked samples were determined by comparing the peak height obtained from freshly prepared sample extracts at low, medium and high concentration levels. The recoveries of teicoplanin were found to be >90% ($n = 3$).

3.2. Chromatogram of each teicoplanin component

The conditions and procedures described in this report are suitable for clinical monitoring of teicoplanin in CSF. The amount of biological constituents is less in CSF than in plasma, and theoretically, CSF sample can be assayed directly without extraction. However, the biological constituents may damage the electrochemical detector; therefore, we filtered the CSF sample using Millex-HV. The standard peaks did not change significantly before and after filtration in terms of and peak height (Table 1).

Table 1
Comparison of peak height before and after filtration with Millex-HV (Millipore, MA, USA)

Filtration	*N*	Peak height
Before	3	15319 ± 1370
After	3	15023 ± 1030
Index of correlation		0.9694

10 μL of teicoplanin (40 μg/mL).

Typical chromatograms of teicoplanin obtained under the above mentioned conditions are shown in Fig. 3. A chromatogram of the standard solution revealed six major peaks, and they correspond to A3-1 (hydrolyzed component), A2-1, A2-2, A2-3, A2-4 and A2-5 components (Fig. 3A). A chromatogram of filtration-extracted CSF sample obtained from a patient administered teicoplanin is shown in Fig. 3B. The separation patterns were almost the same for the standard solution and CSF sample. The plasma concentration–time profile of each teicoplanin component in the liver transplant recipient is shown in Fig. 4.

Both the sensitivity and precision of the method are good, and no interfering peaks are seen in plasma or CSF samples. The simple and reliable liquid chromatographic method described is suitable for clinical monitoring of plasma or CSF teicoplanin levels in patients with meningeal infection.

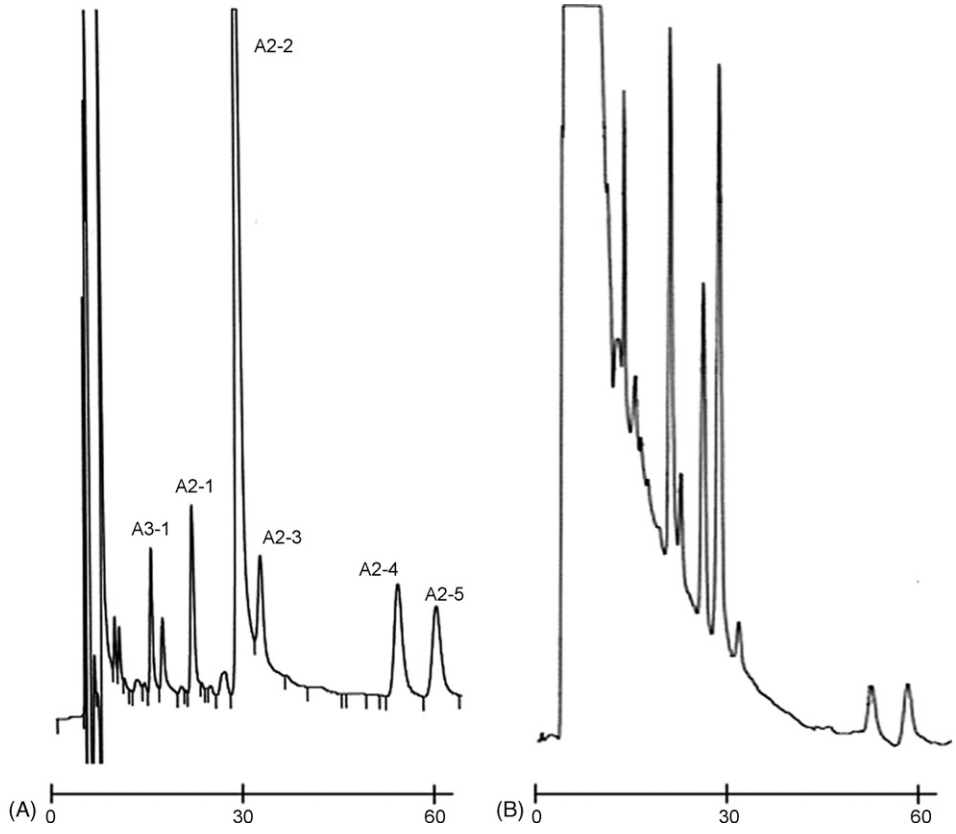

Fig. 3. HPLC chromatograms of teicoplanin standard solution (A) and an filtration-extracted CSF sample (B).

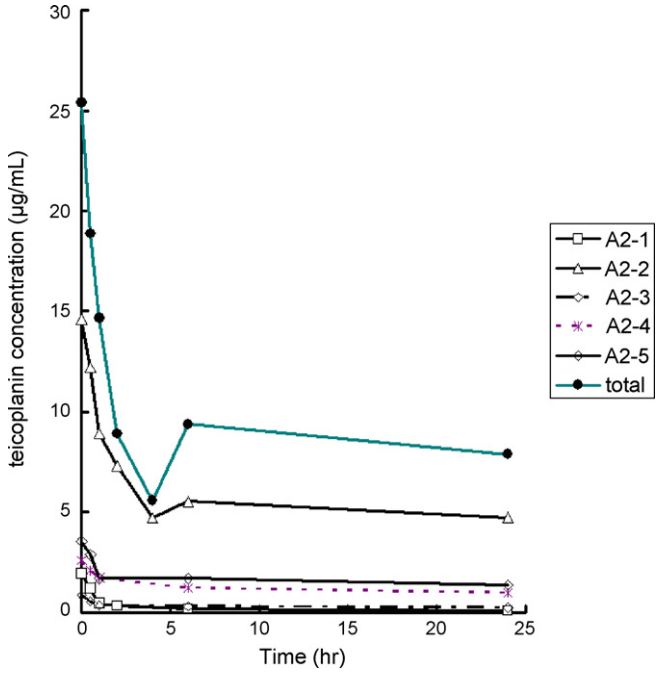

Fig. 4. Concentration–time profile of total teicoplanin and each components in plasma.

Several analytical methods for determining the plasma concentration of teicoplanin have been reported. McCann et al. [7] reported that the limit of quantitation of plasma or serum using an HPLC method with UV detection was 10 μg/mL. On the other hand, Joo and Luthy [4] derivatized teicoplanin with fluorescent and improved the sensitivity compared with UV detection of nonderivatized. Recently, Hanada et al. [9] reported a quantitative determination method for the total unbound concentrations of six teicoplanin components in human plasma by HPLC method with UV detection. It is, however, difficult to use these HPLC methods to determine CSF concentrations because of the low sensitivity or time-consuming procedure. The present assay provides a simple and reliable liquid chromatographic method for the quantitative measurements of individual teicoplanin components. The method described is suitable for clinical monitoring of plasma or CSF levels in patients with meningeal infection and for studies involving pharmacokinetics of individual teicoplanin components.

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