Analysis of tigecycline in cerebrospinal fluid and serum of patients with multidrug-resistant acinetobacter baumannii central nervous system infection by HPLC-MS/MS

Sixun Guo[1], Xina Li[1], Rui Duan[1], Shuo Tian[1], and Xin Hai[1]

[1]Affiliation not available

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Abstract

Abstract Background This study aimed to establish a method to determine tigecycline (TGC) in cerebrospinal fluid (CSF) and serum of 12 patients with Multidrug-resistant acinetobacter baumannii (MDRAB) central nervous system infection (CNSI) and evaluate the correlation of TGC in CSF and serum samples. Methods TGC in CSF and serum was extracted by acetonitrile and detected by HPLC-MS/MS. The separation was performed on the Waters XBridge® BEH Shield RP18 column. This method could achieve the quantification accurately in a very short time. Results For these 12 patients, the trough concentration ranges of TGC in CSF and serum at steady-state were 16.35-53.56 ng/mL and 67.76-211.9 ng/mL. The CSF-to-serum ratio of TGC at steady-state trough concentration was ranged from 21.46% to 44.46%, and the mean value was 31.61 ± 8.13%. The correlation of TGC in CSF and serum was 0.5065. CNSI might have no potential to increase the penetration ability of TGC to CSF. Conclusion The method was validated to be accurate and effective. The correlation between the concentrations of TGC in CSF and serum at steady-state was demonstrated to be positive based on quantification of TGC in bio-samples from 12 MDRAB patients.

1. Introduction

Multidrug-resistant acinetobacter baumannii (MDRAB) is an intractable pathogen which is intrinsically resistant to penicillins, cephalosporins, and fluoroquinolones. The treatment of MDRAB infection is a clinical challenge due to the limit of clinical antibacterial choices [1]. Nosocomial central nervous system infection (CNSI) due to drug-resistant strains had substantially increased over recent years. Owning to multiple drug-resistant and the poor drug penetration through the blood brain barrier (BBB), CNSI caused by MDRAB after neurosurgical procedure could result in longer hospitalization duration, serious neurologic dysfunction and even death[2, 3].

Tigecycline (TGC) is a novel glycyclcycline with broad-spectrum antibiotic activity against multiple pathogens, including Gram-positive pathogenic bacteria, Gram-negative bacteria, atypical pathogens and anaerobe. TGC also plays an important role in the treatment of multidrug-resistant strains infection including vancomycin-resistant enterococci, methicillin-resistant staphylo-cocci, and acinetobacter baumannii. Therefore, TGC is considered as a therapeutic option for MDRAB infection [4, 5]. TGC has been approved for the treatment of complicated skin and skin-structure infections, complicated intra-abdominal infections and community-acquired pneumonia because of the widely distribution in tissue after intravenous infusion[6]. And considering of high sensitivity to MDRAB*in vitro* , TGC is also used for nosocomial pneumonia caused by MDRAB off-label [7].

Generally, the ability of TGC to penetrate through the BBB into cerebrospinal fluid (CSF) was quite weak based on the pharmacokinetic data acquired from health adults without intracranial infection[8]. Poor BBB

permeability limits the use of TGC to treat CNSI caused by MDRAB. However, intracranial diseases such as cerebritis would facilitate the permeability of the BBB[9], and this might increase the CSF-to-serum ratio of TGC. To evaluate the CSF-to-serum ratio of TGC at steady-state when patients were diagnosed with CNSI is important for the treatment of MDRAB CNSI. Up to now, reports to evaluate the penetration of TGC into CSF of patients with CNSI was always based on single case[6, 10]. And some of the reports didn't provide accurate data due to the insensitivity of methods. The lack of data on the ability of TGC to penetrate the BBB of patients with intracranial infection made controversy exist widely about using TGC to treat CNSI caused by MDRAB.

In this study, we established a sensitive, effective and rapid method to analyze TGC in CSF and serum. This method could quantify TGC in CSF or serum ranged from the concentration of 5 ng/mL to 2000 ng/mL in 2.5 min. Depending on this method, concentration of TGC at steady-state in serum and CSF from 12 patients with MDRAB CNSI was determined to evaluate the penetration ratio of TGC into CSF. Correlation of the concentration of TGC in CSF and serum from CNSI patients could be evaluated by quantification of the trough concentration of TGC at steady-state in serum and CSF.

2. Materials and methods

2.1 Chemicals and reagents

TGC (CAT Number: T440015) and internal standard TGC-d9 (CAT Number: 440017) were purchased from Toronto Research Chemicals INC (Toronto, Canada). HPLC-grade and acetonitrile were obtained from Sigma-Alorich (St.Louis, USA). Ammonium formate was provided by Guangfu Fine Chemical Research Institute (Tianjin, China) and formic acid was provided by Kemiou Chemical Reagent Co. Ltd (Tianjin, China).

2.2Standards and quality controls

Stock solution of TGC (1mg/mL) was prepared by dissolving 10 mg TGC in 10 mL deionized water, which was subsequently diluted to a series of concentrations of 0.05, 0.20, 0.50, 1.00, 2.00, 5.00, 10.0 and 20.0 μg/mL as standard solution. A 20 μL of standard solution was added into 180 μL blank serum or CSF to make the serum or CSF calibration at concentration of 5, 20, 50, 100, 200, 500, 1000 and 2000 ng/mL. Three concentration levels (10, 250 and 1800 ng/mL) of quantity control (QC) samples were prepared for analysis of serum and CSF samples in the same way. Standards and QCs were stored at 4°C for no longer than 30 min or frozen at -80degC for long-term storage.

2.3 Sample preparation

A 600 μL acetonitrile and 10 μL IS (TGC-d9, 500 ng/mL) was added in 200 μL serum or CSF. After 1 min vortex and 4000 rpm centrifugation for 5 min, 300 μL supernatant was extracted from samples and then processed by 0.22 μm filter. A 10 μL filtrate was injected into the UHPLC-MS/MS system for analysis.

2.4 UHPLC-MS/MS analytical conditions

The method to analyze TGC in bio-samples has been described in previous review[11]. Relative method was optimized in our study. The chromatographic system was consisted of an AB SCIEX QTRAP® 4500 triple-quadrupole mass-spectrometric system (AB Sciex, MA, USA) coupled with an ultrafast liquid chromatography (AB Sciex, MA, USA). Separation was performed on a Waters XBridge® BEH Shield RP18 column (3.0×50mm, 2.5μm) from Waters Corporation, USA. The temperatures of auto sampler and column oven were 15°C and 40°C respectively. The mobile phase was composed of aqueous mixed with 10 mM ammonium formate (pH=2.5) and acetonitrile according a gradient volume ratio (100:0-10:90-100:0), and pumped at 0.3 mL/min. Quantitative analysis of TGC was processed utilizing positive electron spray ionization in multiple reaction monitoring mode at m/z 586.3-513.2 and 586.3-456.2. TGC-d9 was monitoring in the channel of 595.1-514.2. Mass spectrometer parameters for identification of TGC and TGC-d9 were summarized in Table 1.

2.5 Method and Validation

2.5.1 Specificity and LLOQ

The specificity was evaluated by comparing the chromatograms of samples which were spiked with TGC and TGC-d9 standards in CSF and serum from 6 different individuals. Assessment of LLOQ was depending on the S/N of bio-samples with TGC at the concentration of 5 ng/mL.

2.5.2 Linearity

Blank serum and CSF (180 μL) were added with 20 μL gradient standard solutions at concentrations of 0.05, 0.2, 0.5, 1, 2, 5, 10 and 20 μg/mL respectively to make the TGC content of serum and CSF at the concentration of 5, 20, 50, 100, 200, 500, 1000 and 2000 ng/mL.

2.5.3 Precision and accuracy

Intra-day, inter-day precision were evaluated by analyzing QCs (n=5) at low, medium and high concentrations (10 ng/mL, 400 ng/mL, 800 ng/mL) on the same day or 3 different days respectively. The precision was defined as the relative standard deviation (RSD) of QC sample concentrations determined at 5 replicates. The accuracy was assessed as the percentage to the nominal concentration (%) by analyzing 5 repetitive QCs at each concentration. The mean values should be within 15% of the nominal value except low concentration, which should not deviate more than 20%.

2.5.4 Extraction recovery and matrix effect

The extraction recovery of TGC in serum and CSF was evaluated by comparing the peak area obtained from the blank serum and CSF spiked with analyte at low, medium and high concentrations with which obtained from standard solutions. Blank serum and CSF was processed as stated and then spiked with standard solution at low, medium and high concentrations to assess the matrix effect. The ratio of peak areas of TGC from spiked serum and CSF samples and pure TGC solution was defined as the matrix effect.

2.5.5 Stability

The QCs at multiple concentrations were stored in different condition including room temperature for 2 h, 4°C for 12 h, and -80°C for 60 days were analyzed to evaluate the stability. Freeze-thaw stability of samples was determined by conducting three freeze-thaw circles at low, medium and high concentrations in triplicate. The auto-sampler stability was assessed by reinjection of same sample after 90 min storage in the auto-sampler.

2.6 Application

This study was approved by the ethics committee of the first affiliated hospital of Harbin medical university. 12 patients were diagnosed of MDRAB CNSI (with positive CSF culture of MDRAB for twice) after undergoing a neurosurgical procedure. They were treated with 100 mg TGC as a loading dose, and then the dosage followed the scheme of 50 mg/12h. The CSF samples were collected through lumbar puncture in vacuum collection tube before the start of daily administration after 7 days of treatment. And the blood samples were collected at the same point. Blood samples were centrifuged at 4000 rpm for 5 min (4°C) to separate the serum. The serum and CSF samples were all stored at –80 °C immediately.

3. Results

3.1 UPLC-MS/MS analytical conditions

TGC is a novel glycyclcycline, which belongs to third generation tetracycline. Due to high hydrophilic property (Log P=-1.30) of TGC, hydrophilic columns, such as HILIC and T3 column, were firstly tried to perform the separation. However, the peak shape of TGC provided by hydrophilic columns could not meet our expectation. Waters XBridge® BEH Shield RP18 column (3.0×50mm, 2.5μm) could perform the separation with 100% aqueous phase. The 100% aqueous phase was chosen for the retention of TGC according to the high hydrophilic property of TGC, and TGC could achieve symmetric and sharp peak shapes in this chromatographic condition (Figure 1). Quantification of TGC was performed by MRM in positive mode.

Therefore, formic acid was added in the aqueous phase to enhance signal response. And ammonium formate was utilized to achieve sharp peak of analyte without tailing. TGC-d9 was used as IS to decrease the effect of matrix.

3.2 Samples preparation

Serum and CSF samples were processed with acetonitrile at the volume ratio of 1:3. Compared with diluting residual with mobile phase, the single step protein precipitation with acetonitrile had no significant solvent effect. Therefore, acetonitrile was demonstrated to be protein precipitation solvent to extract TGC from serum and CSF samples, which made the extraction procedure convenient and efficient.

3.2 Method validation

This method performed stable baseline in analytical channel (m/z 586.3-513.2) and IS channel (595.4-514.2). The selectivity was acceptable since there were no interference peak in these channels. The total run time was 2.5 min, which was shorter than most methods to analyze TGC in bio-samples [10, 11]. Respectively, the retention time of TGC in serum samples and in CSF samples was 1.464±0.005 min and 1.466±0.005 min with five continuously reduplicate injections. The chromatograms obtained from blank serum and CSF, blank bio-samples spiked with TGC and IS, and the samples from patients were described in Figure 1. The serum and CSF samples spiked with TGC at lower limit of quantification (LLOQ, 5 ng/mL) also depicted unique and prominent peak at retention time.

Calibration curves were Y=0.019X+0.182 for serum samples (5-2000 ng/mL) and Y=0.0322X+0.53 for CSF (5-2000ng/mL) (a weighting of 1/x was used). Linear coefficients (r^2) were 0.9999 and 0.9997 respectively, which could absolutely achieve accurate samples analysis.

Precision and accuracy data of TGC analysis in serum and CSF samples were tabulated in Table 2. At three QC concentrations of TGC in serum, the accuracy bias was ranged from -4.69% to 3.00%, with the intra-day and inter-day RSD ranged from 2.85% to 7.86%. For the CSF samples at concentration of 10.0 ng/mL, 400 ng/mL and 800 ng/mL, the accuracy expressed in terms of percentage bias was within -1.85% to 6.40%, and the precision varied from 3.06% to 13.72%. The data of matrix effect was shown in Table 3. The IS normalized matrix factors (MFs) for TGC ranged from 79.04% to 109.64% at all levels, and the RSD ranged from 4.93% to 9.43%. All of the data met the requirement of bio-samples quantification.

The results utilized to evaluate the stability of the method were listed in Table 4. There was no significant change of analyte in varied storage conditions within the prescribed time. The accuracy bias of TGC in bio-samples at low, medium and high concentration was ranged from -9.30% to 3.92%, and the RSD was ranged from 2.18% to 8.33%. Simultaneously, the variations of CSF and serum samples spiked with standard solution in auto-sampler for 90 min were acceptable. And the stability of TGC also not affected by three cycles of freezing and thawing significantly.

3.3 Application

The validated method was utilized to quantify the steady-state trough concentration of TGC in serum and CSF samples from 12 MDRAB CNSI patients treated with TGC. The quantification results were listed in Table 5. The concentrations of TGC in CSF and serum were quantified to be 16.35-53.56 ng/mL and 67.76-211.9 ng/mL respectively. The CSF-to-serum ratio was calculated according to the TGC concentration. For these 12 MDRAB CNSI patients, the CSF-to-serum ratio of TGC at steady-state trough concentration was ranged from 21.46% to 44.46%, and the mean value was 31.61 ± 8.13%. To the best of our knowledge, this is the first study to report the CSF-to-serum ratio of TGC at steady-state trough concentration based on samples from 12 MDRAB CNSI patients. This study evaluated the penetration of TGC into CSF for CNSI patient at the steady-state trough concentration. Correlation of TGC in CSF and serum samples from these patients was also evaluated in this study. The correlation curve was showed in Fig.2, and the correlation coefficient (r^2) was 0.5065.

Disscussion

Generally, only small molecular (under the threshold 400-500Da) with high lipid solubility have potential to cross the BBB easily[12, 13]. Lots of effective drugs are restricted to treat neurological diseases because of their inability to penetrate the BBB [14]. CNSI caused by MDRAB has an increasing trend and high mortality. Owing to the drug-resistant ability of MDRAB and barrier function of BBB, few antibiotics are available for the treatment. TGC is known as an antibiotic that has activity against acinetobacter baumannii [15]. The ability of TGC to penetrate BBB is important for the possibility to treat MDRAB CNSI by TGC [16]. TGC is a compound with high water solubility (>100mg/mL) and protein binding rate (71-89%). These decide that TGC has little potential to cross the BBB. However, intracranial diseases such as cerebritis might facilitate the permeability of the BBB. The data about the CSF penetration of TGC is definitely deficient, especially for the patients diagnosed with CNSI. Rodvold *et al* . firstly reported CSF penetration of TGC in humans in 2006. CSF and serum samples were collected from 17 subjects without inflamed meninges within hours after a single 100mg dose of TGC. 11 samples were obtained between 0.92 and 2.1 h, and 6 samples were obtained between 18-24h. CSF-to-serum ratio obtained between 18 and 24 h was ranged from 0.33 to 0.52, which was much higher than those obtained from 0.92 and 2.1 h (0.016–0.095), and the CSF-to-serum $AUC_{0\text{-}24}$ ratio was 0.11. The differences were caused by longer T_{max} and slower clearance velocity of TGC in CSF than that in serum [8]. This study observed poor penetration of TGC in subjects without inflamed meninges. In 2007, Chen *et al.* used 100mg TGC every 12h to treat a patient with sepsis and complicated ventriculitis caused by MDR K. pneumoniae. CSF and blood samples were collected on the 12^{th} day of therapy immediately prior to the scheduled dose of TGC (trough) and 30 minutes after completion of the infusion (peak) to evaluate the BBB penetration ratio of TGC at stay-state. Unfortunately, the analytical method with detectable limit of 0.05 μg/mL, was not able to determine the concentration of TGC in CSF [17]. Ray *et al* . also established an HPLC method with a detectable limit of 0.05 μg/mL to evaluate the CSF-to-serum ratio based on samples from a female patient with meningitis. However, all of the CSF concentrations in this study were below the lower limit of detection. Depending on observed peak area, they estimated the concentration of TGC in CSF was 0.035-0.042 μg/mL, and the penetration ratio of this patient was ranged from 0 to 0.52 [18]. Another case was reported by Pallotto *et al* . in 2014. In this report, a patient with CNSI caused by multidrug-resistant *Enterococcus faecium* was treated by TGC 100 mg intravenous as a loading dose, and followed by 50 mg intravenous every 12 h thereafter. For this patient, CSF-to-serum ratio at peak and trough concentration were 0.066 and 0.106 respectively, and CSF-to-serum $AUC_{0\text{-}24}$ ratio was 0.067. The BBB penetration ratio was significantly lower than that in previous studies[6].

To our knowledge, these three case reports mentioned above were the only studies to describe BBB penetration ratio of CNSI patients. Two of these reports didn't provide accurate data due to the limit of analytical methods. Moreover, the results of these reports were also quite different. Ray *et al* . showed that the TGC CSF concentrations of the patient with CNSI were higher than those of subjects without inflamed meninges in Rodvold's study owing to compromised BBB. On the contrary, the CSF penetration ratio of patient with CNSI in the study of Pallotto were much lower than those of subjects without inflamed meninges in Rodvold's study. Heterogeneity of these cases, such as dosage scheme, sample time and analytical methods, makes it difficult to compare and evaluate these results (Table 6). Consequently, it is important to establish a sensitive method to analyze TGC in CSF of CNSI patients accurately. Moreover, multiple subjects with same dosage scheme and sample time were also important to evaluate the penetration of TGC in CSF of patients with CNSI.

According to our study, the trough concentration of TGC in the CSF at stay-state was ranged from 16.35 to 53.56 ng/mL. The method we established with the LLOQ of 5 ng/mL was sensitive enough to quantify TGC in CSF. This mainly benefited from the high sensitivity of LC-MS/MS. And appropriate chromatographic condition of this method perform a narrow and sharp peak of TGC in 1.47 min, which made the method more sensitive and efficient.

For most results of previous studies, concentration of TGC in CSF was under 50 ng/mL, some researchers proposed that there might be a saturability of TGC to penetrate the BBB. In our study, the positive correlation between the concentrations of TGC in CSF and serum at trough concentration was observed obviously. This indicated that the concentration of TGC in CSF could be affected significantly by the

concentration of TGC in serum, and the saturability of TGC in CSF was not observed at common dosage. The CSF-to-serum ratio of TGC for the patient with CNSI at trough concentration was ranged from 0.21 to 0.44. This result was consisted with the data (0.33-0.52) obtained from adults with non-inflamed meninges in the study of Rodvold *et al* . in 2006. Depending on present study, CNSI did not show the potential to increase the penetration ability of TGC to CSF. Though pathologic states caused by inflammation plays an important role to increase the permeability of BBB, other factors, such as up regulation of efflux transporters at minor damage and high protein binding rate of some drugs, also might restrict the permeation of drugs[13].

5. Limitation

In this study, we have evaluated CSF-to-serum ratio of TGC at steady-state trough concentration based on bio-samples from 12 patients with CNSI. For lots of drugs, previous studies has demonstrated that the peak time and clearance time in brain were longer than that in plasma[19, 20]. This might lead to the difference of CSF-to-serum ratio at different periods [8]. Therefore, the evaluation of penetration ability of TGC through BBB based on CSF-to-serum ratio at single period might be influenced by above factors. Increasing of the sample collection points would made the study more potent. And in our study, we compared our results with the data of 6 healthy adults reported by Rodvold [8]. We should not ignore the possible influence of different dosage scheme and sample time occurred in these two studies. However, collection of CSF samples was quite invasive, which makes CSF samples from health human and multipoint collection could not be achieved.

6. Conclusion

In this study, we have established and validated a method to determine the concentration of TGC in serum and CSF. This method could achieve the quantification accurately in 2.5 min. We quantified the bio-samples from 12 MDRAB CNSI patients treated with TGC to evaluate CSF-to-serum ratio of TGC at steady-state trough concentration. And the correlation between the concentrations of TGC in CSF and serum at steady-state was demonstrated to be positive. CNSI also did not show the potential to increase the penetration ability of TGC to CSF.

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Data availability statement

All relevant data are within the manuscript.

Reference

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