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Original article

Pharmacokinetics of arbekacin in bronchial epithelial lining fluid of healthy volunteers

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ABSTRACT

Introduction: Arbekacin is a unique aminoglycoside antibiotic with anti-methicillin-resistant *Staphylococcus aureus* activity. The efficacy of aminoglycosides is related to their serum maximum concentration. Local concentration of antibiotics in pulmonary epithelial lining fluid, rather than its serum concentration, can help determine its clinical efficacy more precisely for treatment of respiratory infectious disease. The objective of this study was to sequentially measure arbekacin concentration in epithelial lining fluid after infusion of a single clinically available dose.

Method: After the initial blood sampling, arbekacin was intravenously infused into 6 healthy volunteers over 1 h. Epithelial lining fluid and serum samples were collected by bronchoscopic microsampling 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after the start of 200 mg arbekacin infusion.

Results: Each probe sampled 10.1 ± 5.2 μl bronchial epithelial lining fluid. The sample dilution factor was 266.7 ± 157.1. Drug concentration was successfully measured in all but 2 of the epithelial lining fluid samples. The maximum concentration of arbekacin in epithelial lining fluid and serum was 10.4 ± 1.9 μg/ml and 26.0 ± 12.2 μg/ml, respectively. The ratio of the maximum drug concentration in the epithelial lining fluid to that in the serum was 0.47 ± 0.19.

Conclusions: The maximum concentration of epithelial lining fluid reached levels that would effectively treat most clinical strains of methicillin-resistant *S. aureus*.

1. Introduction

Until recently, pneumonia has been classified into one of 2 groups: community-acquired pneumonia or nosocomial pneumonia. Now, nosocomial pneumonia is often divided into several categories, including healthcare-associated pneumonia, nursing and healthcare-associated pneumonia, and ventilator-associated pneumonia [1]. One of the major causative microorganisms of pneumonia in these categories is methicillin-resistant *Staphylococcus aureus* (MRSA), which accounts for 3.5–64.4% of cases [2,3].

There are some anti-MRSA agents available for the treatment of infectious diseases that are due to MRSA, including vancomycin (VCM), teicoplanin, linezolid, and daptomycin. In 2006, the Clinical and Laboratory Standards Institute (CLSI) changed the clinical breakpoints of VCM against MRSA in response to increasing rates of treatment failure with previously designated breakpoints [4]. Among anti-MRSA drugs, VCM is the most widely used as primary treatment of MRSA-related disease. However, alternatives are needed because of the recent increase in minimum inhibitory concentrations (MIC) of VCM against MRSA, also known as MIC creep [5]. Given the current decreasing trend in new approval of antibiotics by the FDA, it is important to focus on existing antibiotics.

Arbekacin (ABK), developed in Japan, is unique among aminoglycosides (AGs). This compound has anti-MRSA activity in addition to its ordinary antibiotic spectrum for gram-negative bacteria [6].

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The therapeutic effectiveness of AGs are directly related to their peak concentration, while dose-dependent adverse effects are closely related to their trough concentration and are not necessarily related to peak concentration [7]. The population pharmacokinetics of ABK in Japanese patients, concentration-response relationships for ABK, and optimal concentration targets of ABK for both efficacy and safety have been reported previously [7,8].

The local concentration of the treatment compound at infection sites is an important factor in antibiotic treatment [9]. For pulmonary infections, the intraluminal concentration of the active form of the treatment compound is directly related to its effectiveness [10,11]. Intravenously administered compounds must pass through pulmonary capillary epithelial layers and interstitial space before reaching the epithelial lining fluid (ELF) [10]. Therefore, it is important to determine the concentration of treatment compounds in the ELF. In the treatment of pneumonia, it is imperative that the concentration of the treatment compound in the ELF be greater than the MIC for the target organism. However, the precise peak concentration of ABK in ELF is uncertain. The penetration ratio of other AGs is reported to be 32–70% [12,13], but the penetration ratio for ABK has not yet been reported. We therefore investigated the penetration ratio of ABK by measuring the ABK concentration in ELF sampled using the bronchoscopic microsampling (BMS) method. We used BMS to sample bronchial ELF directly from the surface of bronchi using a polyester fiber rod and then measured the concentration of antibiotics in the sampled ELF [14–18].

2. Method

2.1. Study design and subjects

The present study was a prospective, nonblinded study of the concentration profiles of ABK in bronchial ELF and serum of healthy adults. The study was conducted for 6 healthy, nonsmoking adult volunteers who had no clinical illness in the 2 weeks prior to the study and no history of other significant diseases. All study protocols were approved by the institutional ethics committee of the Keio University School of Medicine and written informed consent was obtained from each subject before entry into the study.

2.2. Bronchoscopic microsampling (BMS)

Each subject received an intravenous infusion of a single 200 mg dose of ABK diluted into 100 ml of normal saline that was delivered in a 1 h period. Using a BMS probe under bronchoscopy, ELF samples were collected 1, 1.5, 2, 2.5, 3, 4, 5 and 6 h after the start of ABK infusion. The BMS sampling schedule is shown in Fig. 1.

After local anesthesia using 4% liquid lidocaine, a BC-401C BMS probe (Olympus Medical Systems, Tokyo, Japan) was inserted through the working channel of a flexible fiber optic bronchoscope into a sub-sub-segmental bronchus of the right lower lobe, B8. The inner probe was then advanced into the distal airway, and bronchial ELF was sampled by gently placing the probe at the target site on the bronchial wall for 10s. The wet inner probe was sectioned 3 cm from the tip. Probes were weighed after placing them in a pre-weighed tube. Samples were diluted by adding 2 ml of saline to the tube and vortexing for 1 min. The solution was transferred to a new tube and was stored at −80 °C until analysis. The probe was then dried and weighed again to determine the volume of ELF recovered.

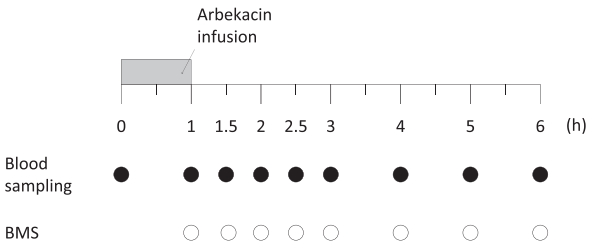

Fig. 1. Sampling schedule for bronchoscopic microsampling of epithelial lining fluid and blood arbekacin was infused over 1 h period. Each filled circle was the time when blood sampling was performed. Open circle was the time when BMS was performed. BMS; bronchoscopic microsampling.

2.3. Measurement of ABK

Arbekacin sulfate concentrations in ELF were measured using a liquid chromatograph (Agilent 1200 Series SL, Agilent Technologies, Inc., California, USA) coupled with a tandem mass spectrometer (API5000, AB Sciex, Massachusetts, USA). Gentamicin sulfate (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was used as an internal standard at a concentration of 4000 ng/ml in water.

Each ELF sample (100 μl) was transferred to a tube, and the internal standard solution (20 μl), water (20 μl) and methanol (200 μl) were added and then votex-mixed. The mixture was centrifuged at 20,000 × g for 5 min at 4 °C. The supernatant (150 μl) was transferred to another tube, and water/pentafluoropropionic acid (1000:1, v/v, 300 μl) was added and then votex-mixed. The mixture was injected into the liquid chromatograph–tandem mass spectrometer.

Arbekacin sulfate was chromatographically separated on an analytical column (InertSustain C18, 50 mm length × 2.1 mm inner diameter, 3 μm particle size) using a gradient of water/pentafluoropropionic acid (1000:1, v/v) – methanol/pentafluoropropionic acid (1000:1, v/v) as the mobile phase at 50 °C.

The tandem mass spectrometer was operated in positive-ion mode using the Turbo-Spray interface. Arbekacin was monitored as the precursor ion at 553 m/z and the product ion at 163 m/z. Gentamicin (internal standard) was monitored as the precursor ion at 478 m/z and the product ion at 322 m/z.

The lower limit of quantification of the present assay method was 2.5 ng/ml. The coefficient of variation for quality control ELF samples at the lower limit of quantification (2.5 ng/ml, $n = 3$) of Arbekacin sulfate was 12.4%. The range of accuracy of the assay method was 84.8%–108.0% at the lower limit of quantification (2.5 ng/ml, $n = 3$).

Because the ELF sampled using BMS was diluted with 2 ml of saline, the concentration of ABK in bronchial ELF ($C_{br\text{-}ELF}$) was calculated as

$$C_{br\text{-}ELF} = C_{BMS} \times (2 + V_{br\text{-}ELF})/V_{br\text{-}ELF}$$ [18],

where C_{BMS} is the measured concentration of ABK in the saline-diluted sample and $V_{br\text{-}ELF}$ is the volume of bronchial ELF recovered by the BMS probe.

2.4. Blood samples

Blood samples were collected just as the ABK infusion finished and each time BMS was performed (Fig. 1). Samples were preserved on ice until the last bronchoscopy procedure. Serum was separated by centrifugation at 3500 rpm for 15 min and was frozen until assayed Serum ABK concentrations were determined by fluorescence polarization immunoassay (FPIA).

Blood samples differ from ELF samples in amount of sample. Therefore we chose another way of assay method in measurement of ELF and blood concentration. There are good linear correlations between the FPIA and the High Performance Liquid Chromatography (HPLC) method [19,20].