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Simultaneous Retrodialysis by Calibrator for Rapid *In Vivo* Recovery Determination in Target Site Microdialysis

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ABSTRACT

Concentrations in the interstitial tissue space are of clinical interest for many antibiotics and can be directly measured by microdialysis. Quantitative microdialysis strongly depends on reliable recovery estimates obtained from a suitable calibrator. Cefazolin (CFZ) is frequently used as a prophylactic antibiotic to prevent surgical site infections. This study aimed to develop a reliable and rapid calibration technique for CFZ microdialysis using cefuroxime (CFR) as a calibrator, which is applied simultaneously in the opposite direction via retrodialysis. Liquid chromatography-tandem mass spectrometry method was used for the measurement of both CFZ and CFR in microdialysate. Results from *in vitro* microdialysis experiments confirmed that CFR does not interfere with physicochemical properties of CFZ, and the loss of CFR is proportional to the gain of CFZ in microdialysis studies. Therefore, the validated bioanalytical assay is suitable to be applied in clinical microdialysis study of CFZ where microdialysis probes are simultaneously calibrated by retrodialysis of CFR. This approach shortens the overall sampling time of *in vivo* microdialysis studies significantly since calibration and sampling can be performed simultaneously and not in sequence as usually done. It also eliminates the necessary washout period if probe calibration is carried out before the actual sampling time.

Introduction

Surgical site infections are infections that occur after surgery causing increased morbidity and prolonged patient hospitalization.[1-3] Adequate perioperative administration of prophylactic antibiotics is critical for protecting patients from surgical site infections with cefazolin (CFZ) being one of the first line choices for various surgeries.[4] For an antibiotic to effectively eradicate an infection, the antibiotic should be present in optimal concentrations at the target site for a sufficient period. Because most pharmacologic events occur in the tissues, achieving and maintaining ideal tissue concentrations of prophylactic CFZ near the surgical site is of paramount importance for clinical efficacy. Traditional tissue homogenization analysis is incapable of continuous sampling and can only provide limited information of drug partition in interstitial tissue space, the histologic compartment most closely related to the site of action.[5] In contrast, microdialysis has been used prevalently in clinical pharmacokinetic studies to continuously monitor unbound drug concentrations in the interstitial fluid of virtually every tissue in human.[6]

This minimally invasive and direct technique is based on passive diffusion across a semipermeable membrane at the tip of a microdialysis probe. In brief, the inlet tubing is continuously perfused with a physiological solution at a low flow rate. After probe implantation, substances present in the extracellular fluid of the tissue filter into the probe and solution from the outlet are collected at specific time intervals for analysis. For most compounds, the equilibrium between extracellular tissue fluid and the perfusion medium is incomplete, resulting in $c_{tissue} > c_{dialysate}$. The factor by which the concentrations are interrelated is termed recovery. In clinical studies, *in vivo* recovery is usually estimated by retrodialysis (RD) method assuming *in vivo* recovery equals *in vivo* loss (Fig. 1a). However, this method can only be performed in the absence of target compound in the extracellular fluid, usually before drug administration.[5] Although used frequently, this procedure is time-consuming since there is an additional washout period after RD sample collection. Another approach that would be more efficient and practical for

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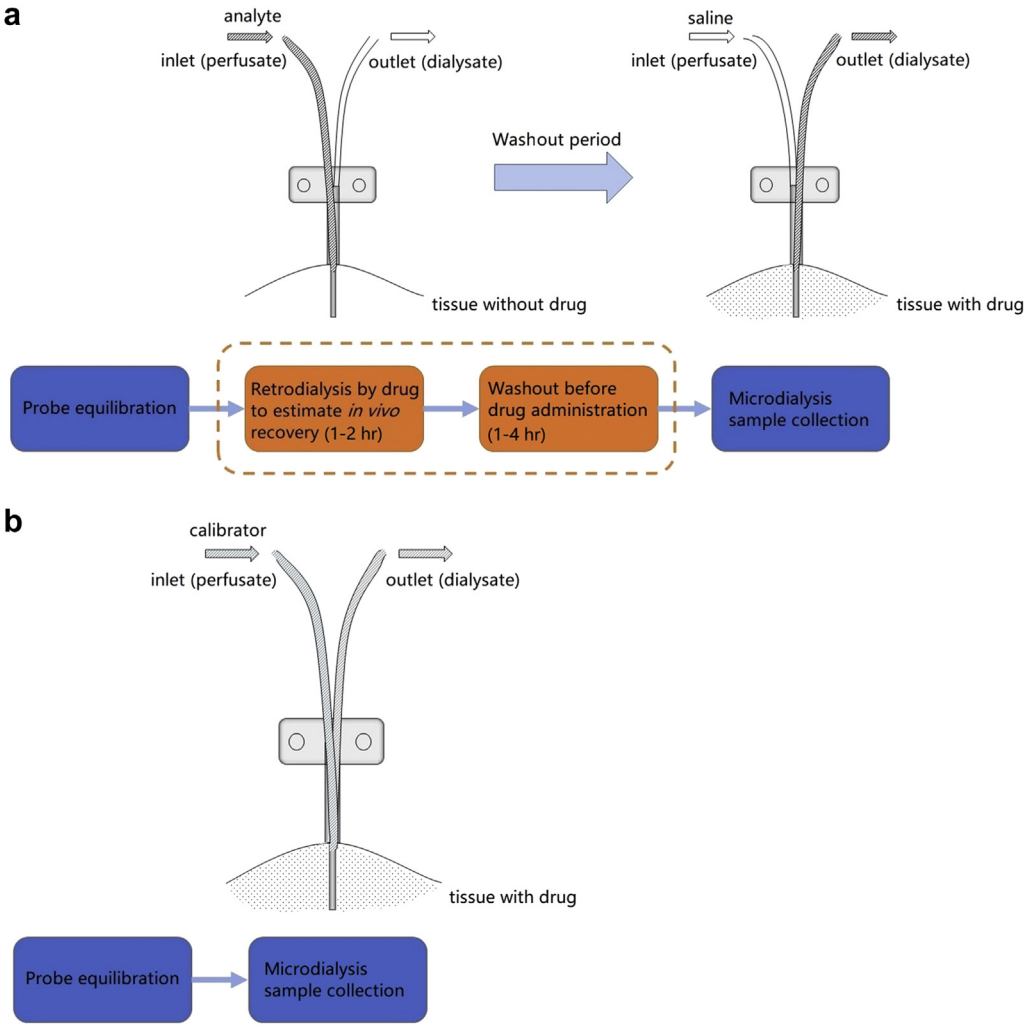

Figure 1. Microdialysis *in vivo* recovery calibration methods. (a) RD by drug; (b) RD by calibrator. The total duration of clinical microdialysis experiments shortens for 2-6 h if microdialysis catheters are simultaneously calibrated via RD by calibrator method.

microdialysis studies during surgery is RD by calibrator (Fig. 1b). With the assumption that relative recovery by the loss of the calibrator is representative for the recovery by a gain of the analyte, *in vivo* recovery of the analyte can be simultaneously estimated from the corresponding recovery of calibrator by a predetermined relative recovery ratio.[7] One of the crucial issue in the application of this method is finding a suitable calibrator that has similar physicochemical property as the analyte of interest but does not interfere with each other. The suitability of calibrator and recovery ratio between analyte and calibrator can be explored from *in vitro* microdialysis experiments.

CFZ, a first-generation cephalosporin antibiotic, is commonly used as one of the prophylactic agents. Previous studies have applied clinical microdialysis technique to evaluate CFZ tissue disposition in skeletal muscle, adipose tissue, and skin.[8-13] Among these, most microdialysis studies applied time-consuming RD method for *in vivo* probe calibration. In this present study, we propose to use cefuroxime (CFR) (a cephalosporin antibiotic chemically similar to CFZ) as a calibrator for microdialysis of CFZ. The aim of this study was to investigate the suitability of CFR as a calibrator for microdialysis of CFZ. To analyze both CFZ and CFR in microdialysates, a sensitive and robust analytical method should be developed and validated. Thus, we here describe an assay for simultaneous determination of CFZ and CFR in microdialysates and report results from *in vitro* microdialysis experiments. These experiments are a prerequisite for the application of microdialysis of CFZ using CFR as calibrator in a planned clinical study.

Materials and Methods

Chemicals and Reagents

CFZ (purity ≥98%), CFR (high-performance liquid chromatography suitable), and internal standard (IS, CFZ-$^{13}C_2$,^{15}N) were purchased from Cayman Chemical (Ann Arbor, MI), Sigma-Aldrich Co. (St. Louis, MO), and Toronto Research Chemicals Inc. (Toronto, Canada), respectively. All 3 compounds are commercially available as the sodium salt. Sodium chloride, liquid chromatography–mass spectrometry (LC-MS)-grade acetonitrile, methanol, and formic acid were purchased from Fisher Scientific (Philadelphia, PA). Triple distilled water was prepared in a Corning Model AG3 Still system and passed through a 0.2-μm Millipore nylon membrane (Millipore, Burlington, MA). Normal saline (0.9% NaCl) was prepared by dissolving sodium chloride into the triple distilled water.