Determination of the Permeability Characteristics of Two Sulfenamide Prodrugs of Linezolid Across Caco-2 Cells

KWAME W. NTI-ADDAE, VICTOR R. GUARINO, GAUTAM DALWADI, VALENTINO J. STELLA

Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, Kansas 66047

Received 19 August 2011; revised 20 October 2011; accepted 31 January 2012

Published online 28 February 2012 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23084

ABSTRACT: The purpose of this work was to study the permeability of two relatively lipophilic sulfenamide prodrugs of linezolid (clog*P* 0.85), N-(phenylthio)linezolid (**1**, clog*P* 2.77) and N-[(2-ethoxycarbonyl)ethylthio]linezolid (**2**, clog*P* 1.43), across Caco-2 cell monolayers. Both prodrugs were found to convert to linezolid in the donor compartment presumably from the reaction with free thiol groups on proteins on the surface of the Caco-2 cells, as no conversion was seen in the donor compartment media per se. Neither of the prodrugs could be detected in the receptor phase from either apical (AP) to basolateral (BL) or BL to AP studies. However, the appearance of linezolid in the receptor phase was biphasic with an initial rapid phase suggesting that the prodrugs were indeed more permeable, and for a short period, some prodrug was able to permeate in competition with conversion to linezolid on the donor phase surface. It appears that the prodrug was able to permeate was rapidly converted to linezolid prior to acceptor phase appearance. The second slower phase was due to the permeability of the donor-phase-formed linezolid, with the slopes similar to those from control experiments with linezolid. The limitations and possible utility of oral sulfenamide prodrugs are discussed. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:3134–3141, 2012

Keywords: sulfenamides; prodrugs; permeability; Caco-2 cells; bioconversion; stability; MDCK cells

INTRODUCTION

The overall objective of this work was to evaluate the permeability characteristics of two lipophilic sulfenamide prodrugs, N-(phenylthio)linezolid (**1**) and N-[(2-ethoxycarbonyl)ethylthio]linezolid (**2**), of the antimicrobial oxazolidinone, linezolid (**L**), across a Caco-2 cell monolayer.[1–7] Linezolid has a clog*P* value of 0.85, whereas the values for **1** and **2** are 2.77 and 1.43, respectively. Both **1** and **2** would be expected to show greater permeability across the lipid bilayer membranes of Caco-2 cells, provided they are not substrates for an efflux mechanism.

Our goal in developing sulfenamides as prodrugs was to improve the physicochemical properties of amide functional group containing drugs.[8–14] Previous studies have shown that sulfenamides can be successfully used to improve solubility; however, an earlier preliminary study to evaluate the permeability characteristics of lipophilic sulfenamide prodrugs across Madin–Darby canine kidney (MDCK) cell monolayers was consistent with rapid degradation of the prodrugs in the donor phase, resulting in no detectable prodrug concentration in the receiver compartments of the apical (AP) to basolateral (BL) directional study and vice versa.[14] A published study of more lipophilic sulfenamide prodrugs of metformin showed some improvement on oral dosing, but the effects were modest (Fig. 1).[15]

MATERIALS AND METHODS

Solvents and Chemicals

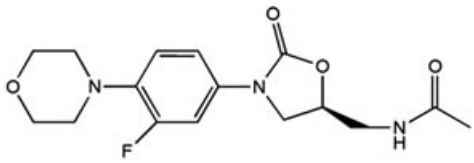
Fetal bovine serum (FBS, heat inactivated), buffered Dulbecco's modified Eagle's medium (DMEM; high

Correspondence to: Valentino J. Stella (Telephone: +785-864-3755; Fax: +785-864-5736; E-mail: stella@ku.edu)

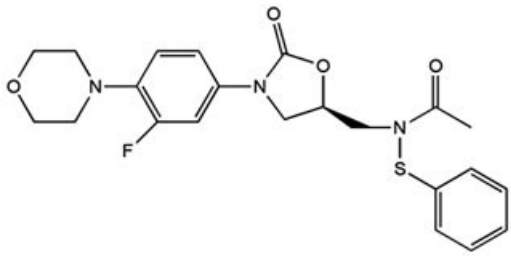
Kwame W. Nti-Addae's present address is Materials Discovery and Characterization, Pharmaceutical Development, Vertex Pharmaceuticals, Cambridge, Massachusetts 02139.

Victor R. Guarino's present address is Pharmaceutical Candidate Optimization, Bristol–Myers Squibb, Princeton, New Jersey 08543-4000.

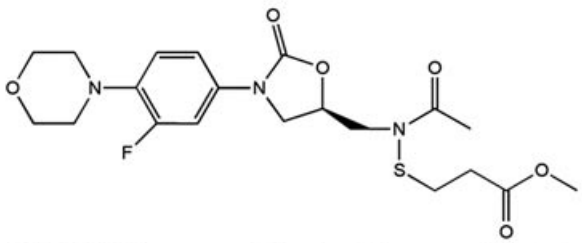
Journal of Pharmaceutical Sciences, Vol. 101, 3134–3141 (2012)



(*S*)-*N*-((3-(3-fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl)methyl)acetamide
[Linezolid, **L**]



(*R*)-*N*-((3-(3-fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl)methyl)-*N*-(phenylthio)acetamide
[N-(phenylthio)linezolid, **1**]



(*R*)-methyl 3-(*N*-((3-(3-fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl)methyl)acetamidothio)propanoate
[N-((2-ethoxycarbonyl)ethylthio)linezolid, **2**]

Figure 1. Structures of sulfenamide prodrugs of linezolid (**1** and **2)** and linezolid (**L**) used in the study Caco-2 permeability studies.

glucose with L-glutamine, 25 mM Hepes buffer, pyridoxine hydrochloride, without sodium pyruvate), unbuffered DMEM, Hank's balance salt solution (HBSS), Dulbecco's phosphate buffer solution (PBS), nonessential amino acid, trypsin [with 0.25% ethylenediaminetetraacetic acid (EDTA)], and penicillin–streptomycin (10,000 IU penicillin/ 10,000 μg/mL streptomycin) were purchased from Mediatech Inc. (Manassas, Virginia). Transwell filter plates (12 well, 0.3 μm pore size) and T-75 flasks were purchased from Fisher Scientific Inc. (Pittsburg, Pennsylvania). Caco-2 cells were obtained from the laboratory of Professor Ken Audus at the University of Kansas (Lawrence, Kansas).

Analyses were carried out on an HP 1050 LC system operated with Agilent Chem Station Software. A Zorbax C18 (5 mm by 2.1 mm, 5 μm particle size) column was used for high-performance liquid chromatography (HPLC) analysis. Ultraviolet detection was at 258 nm and the method used is as described earlier.[12]

Caco-2 Permeability Assay

Media Preparation

A 10% FBS–DMEM Caco-2 media was used for the culture of all Caco-2 cells. To prepare 500 mL of the FBS–DMEM Caco-2 media solution, 50 mL of FBS (heat inactivated, 10%) was filtered through 500 mL cellulose acetate filter with 5.5 mL nonessential amino acids (1%) and 5 μL of 100 units of penicillin/100 μg/mL streptomycin (1%). A 190 mL aliquot of buffered DMEM was added, followed by 250 mL of unbuffered DMEM to give a final volume of 500 mL.

Cell Culture

A cryogenic tube containing Caco-2 cells was placed in a water bath at 37°C to thaw the cells. After several minutes, the cryogenic tube containing the thawed cells was sterilized in 70% ethanol before pipetting cells into a T-75 flask containing 13 mL of Caco-2 media. The cells were grown in an incubator at 37°C in an atmosphere of 5% CO_2. The media were changed