ELSEVIER

International Journal of Antimicrobial Agents 29 (2007) 212–216

INTERNATIONAL JOURNAL OF Antimicrobial Agents

www.ischemo.org

Short communication

Evaluation of an intracellular pharmacokinetic in vitro infection model as a tool to assess tuberculosis therapy

Diane M. Cappelletty *

The University of Toledo, College of Pharmacy #609, 2801 W. Bancroft Street, Toledo, OH 43606, USA

Received 28 March 2006; accepted 3 October 2006

Abstract

In vitro intracellular infection models have been used to evaluate drug therapy against *Mycobacterium tuberculosis*; however, they do not simulate human pharmacokinetics. This study demonstrates the intracellular and extracellular killing activity of antimycobacterial drugs in a pharmacokinetic intracellular in vitro model. The pharmacokinetic parameters of levofloxacin, rifampicin and isoniazid were controlled in the central chamber; drug passively diffused into the cell culture inserts and then into the macrophages. In the pharmacokinetic model, the rates of killing observed were faster than other in vitro methods and allowed for a higher initial inoculum to be utilised. The pharmacokinetic model more closely mimics in vivo conditions than other in vitro systems and is a new instrument for evaluating the activity of antimycobacterial agents.

Keywords: Intracellular; *Mycobacterium tuberculosis*; Pharmacokinetic model; Isoniazid; Rifampicin; Levofloxacin

1. Introduction

Mycobacterium tuberculosis (MTB) grows intracellularly and extracellularly and causes infections that require the use of multiple drug therapy to kill the intracellular organisms effectively and to prevent the emergence of resistant organisms. Whilst extracellular models exist, satisfactory intracellular models are lacking. Therefore, there is a need to develop better pharmacokinetic and pharmacodynamic intracellular infection models to evaluate drug therapy against MTB. In vitro macrophage models are being employed more frequently for the screening of agents for antimycobacterial activity. However, the current models have a limited ability to evaluate regimens for therapeutic efficacy [1–7]. The first method involved exposure of infected macrophages continuously to various static drug concentrations followed by assessment of the intracellular viability of organisms [1–5]. In this method, an evaluation of the killing activity of peak concentrations of antitubercular drugs against MTB in murine macrophages was determined [3]. Therefore, these studies addressed the killing activity of continuous infusion of antimycobacterial agents and not intermittent daily administration of drugs as they are used clinically. The second method exposed macrophages to daily 2 h pulses of achievable peak concentrations of drugs, following which the antibiotic-containing medium was removed and replaced with antibiotic-free medium [6,7]. The latter method of drug exposure is closer to in vivo conditions, however it does not provide the complete time–drug concentration profile that is achievable in an in vitro pharmacokinetic model. The murine model has been the primary tool for screening agents for activity against mycobacteria and for evaluating therapeutic regimens against MTB. Limitations or problems with the murine model include: (i) the number of mice required for studies, which averages approximately six to eight mice per group plus early and late controls; (ii) the cost of maintaining these animals prior to and during the study; and (iii) the pharmacokinetic properties of the drugs in the murine model are not the same as in humans, therefore the effect observed in mice may not accurately predict the effect obtained in humans. The objective of this study was to establish a new pharmacokinetic/pharmacodynamic intracellular and extracellular infection model for tuberculosis.

* Tel.: +1 419 530 1957; fax: +1 419 530 1950.
E-mail address: dcappel@utnet.utoledo.edu.

0924-8579/$ – see front matter © 2006 Elsevier B.V. and the International Society of Chemotherapy. All rights reserved.
doi:10.1016/j.ijantimicag.2006.10.001

2. Methods

The following monotherapies were simulated: isoniazid every 24 h (q24h), peak concentration 4.5 μg/mL and half-life 2 h; levofloxacin q24h, peak concentration 7 μg/mL and half-life 6 h; and rifampicin q24h, peak concentration 7 μg/mL and half-life 3 h.

Mycobacterium tuberculosis H37Rv, a standard laboratory strain susceptible to all agents, was obtained from the American Type Culture Collection (ATCC) and was used for all experiments. The organisms were grown in Middlebrook 7H9 broth to a density of ca. 10^8 colony-forming units (CFU)/mL. Aliquots of the organism suspension were frozen at −80 °C until needed for infecting macrophages.

Human monocyte-like cells (THP-1) obtained from ATCC were used for all experiments. They were maintained with RPMI-1640 media containing 5% fetal bovine serum (FBS) at 37 °C and 5% CO_2. The THP-1 cells (10^8 cells/mL) were mixed with 1 mL of organisms that had been thawed and diluted to ca. 10^6 CFU/mL. The organism/cell suspension was incubated at 37 °C for 2 h to allow phagocytosis to occur. The cells were washed four times with sterile phosphate-buffered saline (PBS) to remove the extracellular organisms. The infected THP-1 cells were diluted in RPMI-1640/FBS medium to ca. 5×10^4 cells/mL and ca. 10^6 organisms/mL and then incubated overnight. Four millilitres of the infected cell suspension was added to the cell culture inserts for all model experiments.

A schematic depiction of the intracellular model, which was adapted from Hultén et al. [8], is shown in Fig. 1. The central compartment is a glass chamber with input and output ports on the side of the chamber. Drugs were injected into the central compartment through a port on the top of model. A magnet placed in the central compartment continuously mixes the drug and culture medium. Fresh cell culture medium (RPMI-1640 with 5% FBS) was pumped into the central compartment with a peristaltic pump at a rate to achieve the desired half-life of each antimycobacterial agent. Drug-containing medium was pumped out of the central compartment and collected in a closed flask containing bleach. Cell culture inserts 24 mm in diameter with a 0.45 μm polyethylene terephthalate (PET) membrane were used to contain the infected THP-1 cells. Six cell culture inserts were placed in openings in a stainless-steel rack and the rack stood inside the central compartment. The entire model apparatus was placed inside a CO_2 incubator to achieve optimal cell culture conditions for the duration of the experiments. All experiments were performed in duplicate and colony counts were performed on Days 0, 1, 2, 3, 5 and 7.

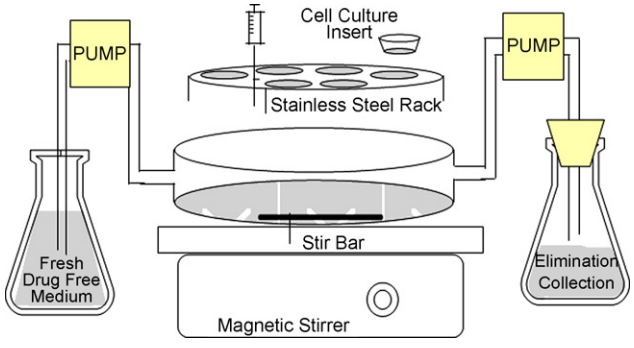

Fig. 1. Schematic representation of the pharmacokinetic intracellular model.

The THP-1 cells were not attached to the membrane but remained in suspension and the membrane was scraped to ensure removal of the cells from the inserts. The cell suspension was centrifuged at 800 rpm for 10 min and the supernatant was removed, serially diluted and plated onto 7H10 agar to detect the presence of extracellular organisms. The cell pellet was washed three times with PBS and centrifuged as above. The cell pellet was lysed with 0.25% sodium dodecyl sulfate (SDS) and the reaction was neutralised after 10 min with 5% bovine albumin. The lysate was vortexed for 1 min, serially diluted and plated onto 7H10 agar to determine the viability of the intracellular organisms. The intracellular organism counts were reported as CFU/mL of cell suspension and the extracellular organism counts were reported as CFU/mL of fluid. All plates were incubated at 37 °C in 5% CO_2 for 21–28 days before colony counts were performed. SDS without neutralisation by bovine albumin decreased colony counts by 9%, and in the presence of bovine albumin SDS had no impact on organism viability.

Samples (1 mL) were removed from the central compartment over the duration of the experiment and were analysed to ensure that the desired half-life and peak concentration of the drugs were achieved. Bioassay was utilised for determination of rifampicin and levofloxacin concentrations and high-performance liquid chromatography (HPLC) was used for isoniazid concentrations. The levofloxacin bioassay was performed using antibiotic medium number 5 and *Escherichia coli* ATCC 25922 ($r^2 \geq 0.99$; intraplate and interplate variation ≤10%). Antibiotic medium number 1 and *Micrococcus luteus* were used for the rifampicin bioassay ($r^2 \geq 0.99$; intraplate and interplate variation <10%). The isoniazid HPLC assay was linear over a range of 0.5–20 μg/mL ($r^2 \geq 0.99$; intraday and interday precision 1–6% and 6–10%, respectively) [9]. Samples were stored at −80 °C until assayed.

The mean killing results obtained from the rifampicin and levofloxacin monotherapy pharmacokinetic model experiments were compared with the results obtained by Mor et al. [6,7] using a *t*-test, since only the mean results were available from the other two studies. A *P*-value ≤ 0.05 was considered significant.

3. Results

The central chamber peak concentration and half-life of rifampicin were 7.0 ± 0.3 μg/mL and 3.1 ± 0.7 h. For levofloxacin, the achieved peak concentration and half-life were 7.5 ± 0.3 μg/mL and 6.6 ± 0.3 h. The central chamber peak