DR SCOTT W MUELLER (Orcid ID : 0000-0003-1238-8199)

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TITLE PAGE

Ribavirin and Cellular Ribavirin-Triphosphate Concentrations in Blood and Bronchoalveolar Lavage Fluid in Two Lung Transplant Patients with Respiratory Syncytial Virus

Running Title: Ribavirin and iRTP BAL concentrations in RSV

Scott W. Mueller, Pharm.D.[1,2], Tyree H. Kiser, Pharm.D.[1,2], Taylor Morrisette, PharmD[1,2], Martin R. Zamora, MD[3], Dennis M. Lyu, MD[4], Jennifer J. Kiser, Pharm.D., Ph.D.[5]

[1]Department of Clinical Pharmacy, University of Colorado Skaggs School of Pharmacy and Pharmaceutical Sciences, Aurora, CO, United States, [2]Department of Pharmacy, University of Colorado Hospital, Aurora, CO, United States, [3]Department of Medicine-Pulmonary Sciences and Critical Care, University of Colorado School of Medicine, Aurora, CO, United States, [4]Division of Pulmonary and Critical Care Medicine, University of Michigan, Ann Arbor, MI, United States, [5]Department of Pharmaceutical Sciences, University of Colorado Skaggs School of Pharmacy and Pharmaceutical Sciences, Aurora, CO, United States

Corresponding Author: Scott W. Mueller, Pharm.D.; 12850 Montview Avenue, Aurora, CO 80045; Telephone: (303)-724-6470; Fax: (303)-724-0979; Email: Scott.Mueller@cuanschutz.edu

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ABSTRACT

Respiratory syncytial virus (RSV) is responsible for significant morbidity and mortality in the lung transplant population. Oral and aerosolized ribavirin may improve outcomes in lung transplant patients with RSV; however, data relating ribavirin concentrations in plasma and intracellular ribavirin triphosphate (iRTP) concentrations in blood and bronchoalveolar lavage (BAL) fluid cells with efficacy and safety are lacking. We describe ribavirin and iRTP concentrations within various compartments in two adult lung transplant recipients with RSV who were sampled throughout successful treatment courses with oral and inhaled ribavirin. In patient one, iRTP BAL concentrations decreased by 45% over three days after changing inhaled ribavirin to oral (6.32 to 3.43 pmol/10^6 cells). In patient two, iRTP BAL concentrations were 103 pmol/10^6 cells after five days of oral followed by five days of inhaled ribavirin. Further study is needed to describe ribavirin pharmacokinetics in the respiratory compartment to inform clinical use of ribavirin for respiratory viruses.

Key Words: Lung transplant, ribavirin, ribavirin-triphosphate, respiratory syncytial virus, concentration

BRIEF REPORT

INTRODUCTION:

Respiratory syncytial virus (RSV) is a well-recognized pathogen in the lung transplant population and is responsible for significant morbidity and mortality.[1-4] Optimal treatment of RSV in lung transplant recipients remains to be defined; however, ribavirin (oral, intravenous, or inhaled) in combination with steroids and immunoglobulin therapy may have benefits in select patient populations.[1,2] Inhaled ribavirin is costly, requires hospitalization for proper administration, and has not been well-studied in adult lung transplant recipients with RSV.[1,2,4-6] Oral ribavirin may be an alternative for outpatient therapy or for those who cannot tolerate the inhaled formulation of ribavirin. Successful use of oral ribavirin for RSV has been reported; however, small sample sizes, variations in treatment protocols, and inconsistent outcome measures preclude confirmation of efficacy.[1,2,5,7]

Ribavirin is a guanosine nucleoside analog with activity against several DNA and RNA viruses allowing it to be considered as a potential therapy for many viruses.[8,9] Ribavirin undergoes intracellular phosphorylation by host enzymes to a mono- (iRMP), di- (iRDP), and triphosphate (iRTP). The phosphorylated forms of ribavirin have been characterized and associated with efficacy and toxicity in persons with Hepatitis C virus.[10-13] However, studies to define the cellular pharmacology, pharmacokinetics, and pharmacodynamics of oral or inhaled ribavirin for RSV and other viruses are lacking. Through improved understanding of these parameters, ribavirin utilization may be optimized. We report a bedside-to-bench evaluation of ribavirin and intracellular metabolite concentrations in plasma and bronchoalveolar lavage (BAL) samples for two lung transplant patients being treated with sequential oral and inhaled ribavirin for RSV infection.

Methods:

Ribavirin in plasma was measured using a validated high-performance liquid chromatography/ultraviolet method linear in the range of 0.05 μg/mL to 10 μg/mL. Interassay

accuracy for the method was within ±8.2% at all concentrations and precision was ≤12.3%.[14] iRMP, iRDP, iRTP were measured using a validated liquid chromatography tandem mass spectrometry method.[15] The quantifiable linear range for the assay was 0.5 to 200 pmol/sample. Interassay accuracy for the method was within ±8.9% at all concentrations, while precision was ≤7.9%. Intracellular concentrations of metabolites are expressed as pmol/10^6 cells and have been converted to mcg/mL concentrations using mass weights of the metabolites and estimated red blood cells (RBC) and peripheral blood mononuclear cell (PBMC) volumes of 0.1 and 0.2 pL/cell, respectively.[16-19] All samples were obtained using an institutional review board approved protocol (COMIRB #09-0761).

CASE REPORTS:

PATIENT ONE:

A 52-year-old, 90-kilogram lung transplant recipient with confirmed RSV infection by a previous qualitative polymerase chain reaction (PCR) was given one dose of 600 mg oral ribavirin on day one and subsequently was started on 2,000 mg of inhaled ribavirin three times daily due to clinical decompensation which required intubation. A BAL was performed on day two, ten hours after the patient's fifth dose of inhaled ribavirin (Figure 1). Blood and BAL fluid were obtained from our clinical and microbiological laboratory after clinically indicated tests were completed. Plasma ribavirin and BAL iRTP concentrations are reported in table one. On day three, inhaled ribavirin was altered to 600 mg of oral ribavirin three times daily. On day six, a BAL was clinically indicated and performed prior to the morning oral ribavirin administration (trough). Excess plasma pre- and post-oral dose and BAL fluid were again obtained from our laboratory after indicated analyses were completed. Plasma ribavirin and BAL iRTP concentrations are reported in table one. Throughout our analysis, the patient's serum creatinine ranged from 1.30-1.60 mg/dL, corresponding to an estimated creatinine clearance of 60-70 mL/min by Cockroft-Gault equation. The patient survived to discharge to a long-term care facility with improved oxygen saturations of 100% on 40% fraction of inspired oxygen with five centimeters of water positive end-expiratory pressure.

PATIENT TWO:

A 64-year-old, 90.1-kilogram lung transplant recipient with confirmed RSV infection by qualitative PCR failed one day of intravenous immunoglobulin plus palivizumab, three days of high-dose steroids, and five days of outpatient therapy with 600 mg of oral ribavirin three times daily. This patient was subsequently hospitalized two days after oral ribavirin discontinuation for worsening chest congestion, wheezing, and shortness of breath with a persistently positive RSV PCR and an increased oxygen requirement from one to three liters of oxygen by nasal canula. The patient received 2,000 mg of inhaled ribavirin three times daily for five days and high-dose steroids for three days (Figure 1). On day five of inhaled ribavirin therapy (seven days since discontinuation of oral ribavirin), a diagnostic BAL was performed. Excess blood and BAL fluid was assayed for ribavirin, and iRMP, iRDP, iRTP. Measured concentrations are reported in table one. Throughout our analyses, the patient's serum creatinine ranged from 1.50-1.75 mg/dL, corresponding to an estimated creatinine clearance of 40-50 mL/min by Cockroft-Gault equation. The patient clinically responded to therapy reporting decreased chest congestion, shortness of breath and maintained oxygen saturations greater than 90% on two liters of oxygen by nasal canula. The patient was subsequently discharged with a reported negative RSV rapid test five days following inhaled therapy.

DISCUSSION:

We report two lung transplant recipients with clinical findings consistent with RSV supported by PCR confirmation who were treated with oral and inhaled ribavirin with corresponding measurements of drug concentrations recovered from plasma/blood and BAL fluid. Ribavirin is a guanosine analogue and prodrug that inhibits viral DNA and RNA synthesis after undergoing intracellular conversion to its active metabolites iRMP, iRDP, and iRTP.[8,9] Although these phosphorylated metabolites have been associated with ribavirin's *in vitro* antiviral properties, they concentrate in RBCs and are also thought to cause the major dose-limiting toxicity of ribavirin, hemolytic anemia.[8,13] Several studies have determined the intracellular pharmacokinetics of ribavirin in patients with hepatitis C, but to our knowledge, no studies have evaluated the plasma, intracellular concentrations, and BAL fluid of oral and inhaled ribavirin in adult lung transplant patients with RSV.[10-12,20] Without pharmacokinetic data, *in vitro* measurements of effective concentration that gives half maximal response (EC_{50}) minimally inform appropriate dosing. Likewise, an *in vitro* selectivity index describes the potential efficacy

and toxicity only within the cell line assessed and would not predict the high rate of hemolytic anemia observed in human clinical studies for ribavirin to treat Severe Acute Respiratory Syndrome-Coronavirus and Middle East Respiratory Syndrome-Coronavirus.[21-23] Characterizing ribavirin concentrations in plasma, RBCs, PBMCs, and BAL fluid may help guide therapy to enhance efficacy and mitigate toxicity in patients with RSV or other viral illnesses. Although we cannot advocate EC_{50} as an appropriate target, ribavirin pharmacokinetic knowledge may guide therapeutic decisions when EC_{50} is unlikely to be reached at the site of infection by choosing an alternative route of administration, synergistic combination, or an alternative antiviral.

This report has limitations. First, patients received both oral and inhaled ribavirin without an adequate washout period. Sampling of patients only on oral ribavirin and comparing them to patients only on inhaled ribavirin would be ideal, especially when considering intracellular concentrations. We cannot directly assess the distribution of oral ribavirin, iRMP, iRDP, iRTP in the lung. Patient one had only one oral dose followed by five inhaled doses providing a reasonable estimate of acute inhaled iRTP concentrations. Following a switch to oral therapy, BAL iRTP concentrations decreased by 45% in three days, faster than the anticipated systemic half-life of the medication. Patient two had BAL iRTP concentrations 16-fold greater than patient one after five days of oral followed by inhaled ribavirin demonstrating significant accumulation with prolonged therapy. Administration through a mechanical ventilator may have impacted the lung concentrations of patient one. This proof of concept analysis provides evidence that BAL fluid can be analyzed for intracellular ribavirin metabolite concentrations. Though real time therapeutic monitoring is not widely available, broadly characterizing these data should aid clinicians in antiviral regimen choice if EC_{50} can reliably be extrapolated to humans. Second, BAL fluid is a heterogeneous matrix. Visual inspection of cells analyzed from BAL samples revealed most cells to be PBMCs, and not epithelial or syncytial epithelial cells. PBMCs may not adequately reflect intracellular ribavirin and metabolite concentrations in syncytia diseased cells. Cellular ribavirin concentrations were calculated using a correction of 0.1 pL/cell for RBCs and 0.2 pL/cell for PBMCs. Cell volumes are estimates and can vary between individuals and within individuals based on cellular activity with a range often reported between 0.2-0.4 pL/cell.[16-19] Due to the estimated mean cell volumes for each patient, the conversion of pmol/10^6 cells to mcg/mL should be interpreted with caution. We did not measure epithelial lining fluid for ribavirin in either patient, therefore did not utilize a blood urea nitrogen

correction. Finally, the effective intracellular concentrations of iRMP, iRDP, and iRTP for the treatment of RSV in lung transplant patients is not known. This most important limitation is the driver of our analysis in lung transplant patients with RSV, as intracellular concentrations obtained with oral and inhaled ribavirin therapy, and optimal concentrations necessary to inhibit viral replication have not been adequately studied *in vivo* for many viruses. *In vitro* studies using cell culture/plaque inhibition suggest a range of 3-30 mcg/mL for ribavirin to effectively inhibit RSV.[24,25] These values are assay dependent and did not measure intracellular concentrations, but rather the amount of ribavirin added to a known volume.

In summary, ribavirin and cellular iRTP concentrations are provided for two adult lung transplant patients treated with ribavirin for RSV with a successful outcome. These data can help guide clinicians and investigators with ribavirin concentrations that proved effective for the treatment of RSV, while mitigating its dose-limiting toxicities. We believe future analyses defining ribavirin distribution and effective intracellular concentrations will allow for judgements to be made regarding viability of therapy as well as appropriate selection of route and dose for various respiratory viruses. Pharmacokinetic and pharmacodynamic studies are needed to guide therapy and dosing. Antiviral studies evaluating efficacy and safety of oral or inhaled ribavirin as a therapy in the lung transplant population should be designed with pharmacokinetic and pharmacodynamic analyses. A bedside-to-bench approach may help guide ribavirin therapy in patients already deemed candidates for ribavirin therapy.

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Author Contributions

SWM, THK, MRZ, DML, and JJK contributed to the conception and design of the work and acquisition of data; JKK analyzed patient samples for drug concentrations. SWM, TM, THK, MRZ, DML, and JJK contributed to the analysis and interpretation of the data, drafting of work,

revisions, and intellectual content; all authors approved the final version of the manuscript and agree to be accountable for the work.

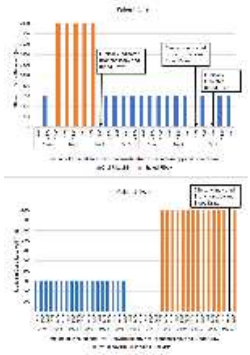
Figure 1: Timeline of ribavirin administrations and excess fluid sampling for respiratory syncytial virus pneumonia in two lung transplant patients

TABLE

Table 1. Ribavirin and Intracellular Metabolite Plasma and Bronchoalveolar Lavage Concentrations

	Plasma Ribavirin Concentration	Blood RBC Intracellular Concentrations	Blood PBMC Intracellular Concentrations	BAL Intracellular Concentrations
Patient One				
First Sample (oral ribavirin followed by inhaled ribavirin)	0.473 mcg/mL (trough)	Not available	Not available	iRTP: 6.32 pmol/10^6 cells (15.3 mcg/mL)
Second Sample (Inhaled ribavirin followed by oral ribavirin)	1.560 mcg/mL (trough) and 2.210 mcg/mL (peak)	Not available	Not available	iRTP: 3.43 pmol/10^6 cells (8.3 mcg/mL)
Patient Two				
First Sample (oral ribavirin followed by inhaled ribavirin)	2.160 mcg/mL (trough)	iRMP: 6.22 pmol/10^6 cells (20.2 mcg/mL) iRDP: 14.3 pmol/10^6 cells (57.8 mcg/mL)	iRMP: 1.17 pmol/10^6 cells (1.9 mcg/mL) iRDP: 0.62 pmol/10^6 cells (1.25 mcg/mL)	iRMP: 851 pmol/10^6 cells (1,379.0 mcg/mL) iRDP: 41 pmol/10^6 cells (82.9 mcg/mL)

		iRTP: 31.3 pmol/10^6 cells (151.5 mcg/mL)	iRTP: 3.89 pmol/10^6 cells (9.42 mcg/mL)	iRTP: 103 pmol/10^6 cells (249.0 mcg/mL)
RBC, red blood cell; PBMC, peripheral blood mononuclear cell; iRMP, iRDP, iRTP, intracellular ribavirin mono-, di-, and tri-phosphate, respectively; pmol, picomole; mcg, microgram, mL, milliliter				



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