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THE UNIVERSITY OF NOTRE DAME AUSTRALIA

Population Pharmacokinetics of Ribavirin in Lung Transplant Recipients and Examination of Current and Alternative Dosing Regimens

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Bachelor of Medicine

A thesis submitted in fulfillment of the requirements for the degree of Masters of Medicine

School of Medicine
The University of Notre Dame
Darlinghurst Campus

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SUMMARY

Ribavirin is used in the treatment of respiratory paramyxovirus infection in lung transplant recipients; however, its pharmacokinetic profile in the transplant population is unknown despite the potential for alterations due to underlying pathology. Furthermore, the ability of current regimens to meet exposure targets has not been established. This study examined the pharmacokinetics of ribavirin in a lung transplant population from which current and alternate dosing regimens were assessed. Population pharmacokinetic modelling was conducted in NONMEM using concentration-time data from 24 lung transplant recipients and 6 healthy volunteers. Monte Carlo simulation was used to assess the ability of dosing regimens to achieve pre-specified target concentrations. A three-compartment model with first order elimination most adequately described ribavirin concentration-time data, with creatinine clearance and patient type (i.e. lung transplant) identified as significant covariates in the model. Simulations indicate that current regimens achieve efficacious concentrations within 24 hours of treatment initiation that increase to supra-therapeutic levels over the treatment period. A regimen of 8 mg/kg q6h oral for 48 hours followed by 8 mg/kg q24h oral for the remainder of the treatment period was predicted to result in >90% of patients exhibiting concentrations within the defined target range throughout the entire treatment course. Additional work to formally establish of target therapeutic concentrations is required; however, this study provides a valuable first step in determining optimal ribavirin treatment regimens for paramyxovirus infections in the lung transplant population.

PUBLICATIONS IN SUPPORT OF THESIS

Manuscripts

Milliken E, de Zwart AES, Alffenaar JC, Marriott DJE, Riezebos-Brilman A, Schteinman A, Evans AM, Glanville AR, Verschuuren EAM, Reuter SE. Population pharmacokinetics of ribavirin in lung transplant recipients and examination of current and alternative dosing regimens. J Antimicrob Chemother; DOI 10.1093/jac/dky466 [Epub ahead of print]

Conference Presentations

Milliken E, Marriott D, Schteinman A, de Zwart A, Sandaradura I, Carlos L, Burrows F, Glanville A, Reuter SE. A predictive pharmacokinetic model of ribavirin plasma concentration in lung transplant recipients [Poster Presentation]. International Congress of the Transplantation Society (TTS), August 2016: Hong Kong.

Milliken E, de Zwart AES, Alffenaar JC, Marriott DJE, Riezebos-Brilman A, Schteinman A, Evans AM, Glanville AR, Verschuuren EAM, Reuter SE. Population pharmacokinetics of ribavirin in lung transplant recipients and examination of current and alternative dosing regimens [Oral Presentation]. International Congress of Therapeutic Drug Monitoring & Clinical Toxicology (IATDMCT), September 2018: Brisbane, Australia.

Milliken E, de Zwart AES, Alffenaar JC, Marriott DJE, Riezebos-Brilman A, Schteinman A, Evans AM, Glanville AR, Verschuuren EAM, Reuter SE. Population pharmacokinetics of ribavirin in lung transplant recipients and examination of current and alternative dosing regimens [Poster Presentation]. Australasian Pharmaceutical Science Association (APSA) Annual Conference, December 2018: Adelaide, Australia.

DECLARATION

I declare that this thesis is an account of my own research and contains as its main content work that has not previously been submitted for an award or degree or diploma in any university or other institution. To the best of my knowledge, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

No competing interests have been identified.

Dr. Eliza Milliken
11th December 2018

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PREFACE

This project was a joint undertaking between St Vincent's Hospital Sydney and the University Medical Centre Groningen, both of which are thoracic transplant centres with established research programs aimed at increasing median survival in solid organ transplant recipients. St Vincent's Hospital Sydney has been a globally recognised centre for excellence in the field of heart and lung transplant since 1987. The Department of Thoracic Medicine has performed over 870 lung transplant procedures since it opened and currently performs over 50 transplants annually. As a global leader in thoracic transplantation the necessity of a multi-disciplinary approach in achieving good outcomes in transplant recipients is well understood. This includes a close working relationship between the Department of Thoracic Medicine and the Department of Infectious Diseases. Infections of all types are a major factor in reduced survival post-transplant due to both acute morbidity and mortality in immunosuppressed patients as well as the immunomodulatory effect of immune/pathogen interface on long-term graft tolerance[1,2,9,10,12,13] Investigation into effective antimicrobial therapies for transplant patients is a primary focus of transplant medicine both at St Vincent's, Groningen and globally.

CHAPTER 1: Introduction

Ribavirin is a guanosine analogue antiviral agent used for the treatment of community acquired respiratory viruses including respiratory syncytial virus (RSV), human metapneumovirus (HMPV) and parainfluenza virus in lung transplant recipients. Ribavirin was licensed for use in 1986 for aerosolised treatment of RSV in infants and the oral formulation was approved for treatment of hepatitis C in 1998, but it has a broad spectrum of activity against DNA and RNA viruses. Until recently the main indication for ribavirin was hepatitis C infection. However with the introduction of direct-acting-antiviral agents ribavirin's primary utility is now respiratory infection in immunosuppressed patients.[1-7] Goals of treatment are both to prevent acute progression to pneumonitis and to reduce the incidence of infection associated bronchiolitis obliterans syndrome (BOS).[5,6] Although historical studies have focused on the efficacy of aerosolised ribavirin, transplant centres now use both intravenous and oral regimes to avoid airborne drug exposure and resultant toxicity to health care staff.[10-14]

Data from studies on lung transplant recipients conducted at St Vincent's Hospital in Sydney support the use of intravenous and oral ribavirin in the treatment of RSV.[14] However, there is no data on the pharmacokinetics of ribavirin in the transplant population, nor comparison regarding therapeutic serum concentrations between different routes of drug administration. Studies from hepatitis C infected patients have been used to establish the safe therapeutic index for ribavirin using the haemoglobin level as a marker of toxicity; however, this data is based on a prolonged oral course of up to six months duration.[15-17,20] Ribavirin is a drug with a long terminal half-life that accumulates in red blood cells, the toxicity therefore may not be similar in short term use, even with identical plasma concentrations. RSV and HMPV have been shown to have an IC50 estimated at 1.35 – 5.82 mg/L *in vitro*.[15-17,20] However, to date, effective short-term dosing strategies for achievement of viral inhibition have not been established in the clinical setting. Current treatment strategies typically comprise a loading dose of 11 mg/kg TDS for the first 24 hours of treatment, followed by a maintenance dose of 10 mg/kg BD with the assumption that maximum time at the highest tolerated plasma concentration will most effectively inhibit viral replication.

It is feasible that transplant recipients as a population may manifest deviations in the pharmacokinetic profile of antimicrobial agents and other drugs due to physiological changes induced by the transplant and subsequent immunosuppressive therapy. Such changes may include: a high incidence of impaired renal function, polypharmacy from immunosuppressive regimens and multiple other routine medications, and heavy corticosteroid exposure with associated alterations in hepatic metabolism. In cystic fibrosis (CF) patients, drug metabolism is altered by abnormal gastrointestinal absorption. Total body fat content and stomach contents also influence the absorption and distribution of ribavirin which may be an issue even in non-CF transplant recipients as the prevalence of malnutrition is high in this population.[15-17,20]

Effective treatment for viral respiratory infection in lung transplant recipients is crucial. However, for the reasons outlined, knowledge regarding effective treatment protocols is lacking. A thorough investigation of the pharmacokinetics of ribavirin in this patient population is required from which effective treatment strategies can be assessed and devised. Initially a review of the literature was undertaken to elicit gaps in the field.

LITERATURE REVIEW

Data Sources

Databases MEDLINE, PubMed and EMBASE were searched using the terms; "ribavirin", "respiratory syncytial virus" or "RSV" and "toxicity" and/or "therapeutic window/index" and/or "serum concentration" and/or "pharmacokinetic*". The Cochrane database was also searched with the same terms.

Publication Selection and Data Extraction

All relevant original studies, meta-analyses, systematic reviews, case-series and case-studies were assessed for inclusion. Reviews and general discussion of community acquired respiratory viruses were not included unless they had specific focus on RSV/HMPV or ribavirin treatment. Reference articles were included if they contained relevant information. Articles that focused exclusively on stem-cell transplantation or immunocompetent paediatric

populations were not included as results from these were not thought to be generalisable to the solid organ transplant population.

Search Results

A search of the Medline database returned a total of 100 articles. The further specifications "English language" and "human" were added reducing the total to 85 articles. Of these 85, 47 were instantly excluded as irrelevant to the topic. A further 17 articles were excluded for being specific only to the immunocompetent paediatric population. A further seven articles were excluded for pertaining specifically to use of ribavirin in the context of stem cell transplant patients. A total of 21 articles from the Medline search were included.

An EMBASE search using the exploded terms " ribavirin", "respiratory syncytial virus" and " transplant" also returned 85 results. Of these, 60 were immediately excluded as irrelevant to the scope of this enquiry. Of the 25 included, an additional four were excluded as duplicated from the Medline search.

A search of the Cochrane database returned two meta-analyses, both pertaining to an immunocompetent paediatric population. These were not included.

Respiratory Syncytial Virus and the Human Metapneumovirus

The threat of community acquired respiratory viruses to transplanted lungs is well described.[21-23] RSV and HMPV cause a spectrum of illness ranging from mild respiratory symptoms to severe lower respiratory tract infections including bronchiolitis, pneumonia and respiratory failure, with a mortality of 10-20%.[24,25] In the setting of lung transplantation, RSV infection has been associated with the development of bronchiolitis obliterans and long-term, irreversible loss of lung function with reduced survival. Current evidence suggests ribavirin reduces the incidence of BOS and the duration of symptoms in lung transplant patients.[6,10-14,21-23] Small case control studies also suggest non-inferiority of oral ribavirin in comparison with intravenous administration for RSV treatment.[5,6,10,12-14]

HMPV produces similar pathology to RSV[25] and has been estimated to be the cause of viral respiratory tract infection in up to 4% of hospitalised adults in the

United States.[27] HMPV is usually a mild self-limiting illness in the general population but causes more serious illness in the immunosuppressed population. RSV is implicated in the development of BOS along with other community acquired respiratory viruses.

Bronchiolitis Obliterans Syndrome

BOS refers to a syndrome of progressive, irreversible airway destruction that afflicts lung transplant recipients.[28] Exact rates of BOS are difficult to determine due to the difficulty of recruiting large cohorts of transplant recipients for study. However, the largest experience, from the International Society for Heart and Lung Transplant (ISHLT) registry, reports that 48% of recipients develop BOS by five years after lung transplant and 76% develop BOS after ten years.[29]

Risk factors for BOS are manyfold. Besides viral infections, other factors that increase BOS risk include episodes of acute immune-mediated organ rejection, bacterial and fungal colonisation, primary graft dysfunction and gastroesophageal reflux disease. Single (rather than double) lung transplant procedure also increases BOS risk.[28] There are emergent theories regarding the pathobiology of BOS. It is postulated that BOS could result from autoimmunity (in addition to allo-immunity) to the usually hidden sub-epithelial collagen type V epitopes. These epitopes become exposed during inflammation from infection or due to ischemia, or vascular reperfusion injury during the transplant procedure.[30] Moreover, increasing evidence suggests that patients with pre-existing antibodies to Human Leucocyte Antigen (HLA) or Major Histocompatibility Complex (MHC) Class I chain-related gene A antigens are at a higher risk of developing BOS after transplantation.[31]

At present no effective evidence-based treatment for BOS is available; the only treatment option is prevention. Severity is based on radiological assessment and ongoing and regular FEV1 measurement in transplant clinics. BOS severity is ranked via a validated international scoring system. The ultimate consequence of BOS is graft failure and death except in the very rare instance of a second transplant.

Clinical Management of RSV Pneumonitis

Oral, intravenous and aerosolised ribavirin have all been administered for RSV infection in lung transplant recipients and other vulnerable populations. There is data supporting the efficacy of all forms of ribavirin for the treatment of RSV pneumonitis in lung transplant recipients.[17] A double-blind trial of placebo versus aerosolised ribavirin on 16 otherwise healthy young adults voluntarily exposed to RSV (and displaying symptoms of respiratory infection) demonstrated diminished viral shedding and a reduced incidence of systemic complaints such as fever and malaise. However, it is notable that it did not have any impact on resolution of respiratory symptoms.[32] Lewis *et. al*. examined aerosolised ribavirin for efficacy against RSV and parainfluenza virus in a cohort of fifteen lung transplant recipients. Only a third of these patients were treated with ribavirin and the results were equivocal.[33] This study also suggests underlying pulmonary fibrosis may be an independent risk factor for worse long-term outcomes despite successful treatment of RSV. However, it could be suggested that patients with pulmonary fibrosis are likely to have less baseline pulmonary reserve. Hynicka and Ensor recommend aerosolised ribavirin as part of a combination antiviral/immunomodulator regime for efficacious treatment of RSV infection.[34] Additionally, studies by Kwak and colleagues and Li *et. al* demonstrated no significant difference in outcomes for oral versus inhaled ribavirin therapy in the context of RSV infection.[35,36] These studies also demonstrated a reduction in dyspnoea and the incidence of BOS after treatment with ribavirin. Kwak notes patients receiving oral ribavirin tended to stay in hospital for longer.[35] However this may be a coincidental correlation as this study had a cohort of only six patients.

Ribavirin Pharmacology

Ribavirin is characterised by multi-compartmental pharmacokinetics with broad distribution into all tissues and a characteristic long terminal half-life. It is a prodrug which is quickly phosphorylated into its active form after crossing the cell membrane via the ubiquitous *es* transporter. However, once phosphorylated it can no longer exit the red cell in the same manner, accounting for the drug's accumulation in erythrocytes and other cells without dephosphorylating enzymes.[15] Approximately 85% of ribavirin is converted into a triphosphate form with the remaining 15% monophosphate or diphosphate

ribavirin molecules.[15] It is postulated that the monophosphate form may be more active against RSV.

The mechanism of anti-viral action is not fully understood, however proposed mechanisms include critical depletion of available guanosine triphosphate (GTP) required for viral DNA/RNA replication. Additionally, the monophosphate form is known to completely inhibit inosine-5'-monophosphate dehydrogenase, an enzyme that cleaves GTP. This mechanism is likely to be most effective in inhibiting paramyxovirus production due to the specific viral replication methods. Ribavirin has also been observed to have immunomodulatory effects on CD4+ cytokine production although this is probably more relevant to the action against hepatitis C.[15,37-43] The anti-viral effect of ribavirin is dependent on dosage and resultant plasma concentrations of the drug; thus understanding the pharmacokinetic properties are critical to the identification of optimal treatment protocols.

Ribavirin Therapeutics

Oral, intravenous and aerosolised ribavirin have all been employed in the treatment of RSV infection; however, evidence for effective plasma concentrations is lacking. Concentrations required to inhibit RSV replication by 50% (IC_{50}) have been estimated at 1.35 – 5.82 mg/L *in vitro*, with similar values for HMPV. Previously publishes reasearch has indicated that effective concentrations are >2.5 mg/L; however, given the known association between plasma concentrations >3.5 mg/L and haemolytic anaemia, a target therapeutic range of 2.5 – 3.0 mg/L is suggested with outer limits of 1.5 – 4.0 mg/L.[44-47] This therapeutic window has been based on observation of patients treated for hepatitis C.[44-47]

As well as the previously discussed dose-associated haemolytic anaemia, ribavirin has been demonstrated to increase the risk of myocardial infarction in patients with pre-existing cardiac disease.[15,48] ribavirin has teratogenic qualities and should not be given to pregnant women or males with pregnant partners.[48] Avoidance of conception is advised for six months after drug exposure which presents particular administration difficulties for health care workers and has resulted in reduced use of the aerosolized form to avoid passive exposure for hospital staff.

Local protocols for treatment of respiratory viruses with ribavirin differ in international centres. This is due to the lack of definitive evidence on superior dosing strategies. Ribavirin's use as an agent for RSV treatment originated in the 1980s, when the aerosolised form, administered inside containment tents on paediatric wards, was recommended for paediatric RSV pneumonitis. However, lack of supporting clinical trials and significant associated toxicity for both patients and health care workers using the drug's aerosolised form lead to the American Academy of Paediatrics withdrawing this recommendation in the early 2000s.[22] In adult patients, ribavirin was extrapolated from its previous paediatric use as a treatment for RSV and other viral causes of pneumonitis and has shown benefit in case series and small cohort studies of immunocompromised patients being treated for RSB in in both PO and IV preperations. [12,21,22]. However, despite common clinical use, the ability of current dosing regimens to meet suggested therapeutic targets is unclear, particularly given the notable physiological differences between solid organ transplant recipients and the general population (with likely to impact on pharmacokinetics). Research to determine ribavirin pharmacokinetics in the lung transplant population and examine the ability of current and alternate dosing regimens to meet the pre-defined therapeutic target is needed.

Aims and Objectives

The aim of this project was to establish the pharmacokinetic profile of ribavirin in lung transplant recipients and to examine dosing strategies to optimise the attainment of target concentrations.

The project was designed to use a population pharmacokinetic approach to address several questions within the scope of the project including, but not limited to:

- Is there a difference between the pharmacokinetics of ribavirin in lung transplant recipients when compared with healthy volunteers?
- Do current dosing strategies reach or exceed the currently accepted safe and effective therapeutic window?
- Do current dosing strategies maintain patients in an effective therapeutic window for the duration of treatment?

- Are there alternative dosing regimens that would better maintain patients at effective plasma concentration for the duration of therapy?
- Is an intravenous loading dose necessary to reach an effective plasma concentration?

Although this was not the primary objective of this particular study, it was envisaged that the developed population pharmacokinetic model could be used for additional work to examine more complex dosing strategies such as opportunities for therapeutic drug monitoring in which dosing would not just be based on generalised population data but personalised for the individual patient.

CHAPTER 2: Methodology

The study comprised a combined prospective and retrospective analysis of real-time pharmacokinetic data from cohorts across two international centres, as well as the inclusion of previously-published ribavirin intensive concentration-time data from healthy volunteers.

ETHICAL CONSIDERATIONS

The study protocols at both clinical centres were reviewed and approved by the respective institutional Human Research Ethics Committees (St Vincent's Hospital Sydney Human Research Ethics Committee HREC/15/SVH/74 and Medisch Ethische Toetsingscommissie University Medical Center Groningen METc 2015/452).

Participants from the prospective (Australian) cohort were recruited via the Lung Transplant Clinic at St Vincent's Hospital. Each study participant was fully informed of the study procedures and was provided with a written information sheet detailing the nature of the study prior to study recruitment. The retrospective cohort (at University Medical Centre Groningen) were not provided with written information as their protocol did not deviate from standard of care. Informed patient consent was obtained under the direction of the UNMCG ethics committee.

The project in entirety was conducted in accordance with the study protocols as approved by the ethics committees and the Declaration of Helsinki.

ELIGIBILITY CRITERIA

To be deemed eligible for study inclusion, potential participants were required to be over 18 years of age, a lung transplant recipient and requiring ribavirin treatment for viral infection. Patients undergoing haemodialysis were excluded from participation. History of previous RSV or respiratory viral infection was not a factor considered in determining eligibility. All levels of severity of infection were accepted into the study including patients admitted to the intensive care unit. No formal restrictions on time since transplantation were included as part of the eligibility criteria, but all patients were required to be

stable since receipt of transplant and not have been transplanted for acute viral pneumonitis.

STUDY PROCEDURES

St Vincent's Hospital

As per standard practice, lung transplant patients who test positive for RSV on qualitative nasopharyngeal swab polymerase chain reaction (PCR) are prescribed a mixed intravenous and oral ribavirin regimen comprising a loading dose of 11 mg/kg TDS IV as a 30-minute infusion for the first 24 hours followed by a maintenance dose of 10 mg/kg BD PO for up to 14 days depending on the duration of clinical symptoms. Clearance swabs are not performed due to the lack of availability of quantitative RSV PCR. Nasopharyngeal swabs remain positive for RSV and HMPV some weeks after resolution of the viral illness due to persistent fragments of dead virus in the airways. ribavirin plasma concentration testing is not performed routinely in this clinical setting.

Upon identification of a suitable patient for study recruitment, the registrar on call for lung transplant patient admissions would alert the study investigator. The study investigator would then assess the patient's suitability for the study, explain the study protocol and goals to the patient and obtain written consent.

Study participants were administered a mixed IV and PO ribavirin dosing regimen as per standard practice. Blood samples for analysis of plasma ribavirin concentrations were collected by the study investigator via venepuncture at various times after dose administration with a rich sampling strategy; the first sample was collected immediately prior to the first dose administration and then at 0.5 hours (after completion of the infusion) and 1, 2, 3, 4, 6 and 8 hours after the start of the infusion. Additional samples were also collected on the morning of Day 4 and Day 7 of treatment immediately prior to the scheduled oral dose and then 2 hours after dose administration.

Due to the logistic difficulties in performing plasma analysis immediately after blood collection a protocol was developed for samples to be centrifuged and separated by the venepuncturist, then frozen to be assayed within working

hours. All investigators were trained in sample preparation by a senior scientist from the Clinical Pharmacology laboratory.

University Medical Centre Groningen

Patients with RSV or HMPV infection are routinely treated with oral ribavirin administered as a loading dose of 11 mg/kg TDS PO for the first 24 hours, followed by a maintenance dose of 10 mg/kg BD PO. Standard of care includes monitoring of plasma ribavirin concentrations at 1.5 hours after administration of the first dose and then immediately prior to morning dose administration on Day 2, 4, 7 and 10. Nasopharyngeal swabs for clearance are not routinely performed.

Study participants were identified from review of patient medical records and data collected retrospectively by the project collaborators.

Biological Sample Analysis

Blood samples were centrifuged immediately after collection, aliquoted for storage and frozen at -20°C for sample stability until ribavirin concentration analysis could occur. Plasma samples were analysed for ribavirin concentrations on dedicated equipment in the Department of Clinical Pharmacology and Toxicology, St Vincent's Hospital and the Department of Clinical Pharmacy and Pharmacology, University Medical Centre Groningen using validated Liquid Chromatography Tandem Mass Spectrometry methodology.[53]

Values for samples returning concentrations below the lower limit of quantification of the assay were not made available by the analytical laboratory; these samples were excluded from the pharmacokinetic dataset.

Healthy Volunteer Dataset

Additional data for the development of the population pharmacokinetic model was extracted from previously published work examining the pharmacokinetics of oral and intravenous ribavirin in healthy normal volunteers.[8] Participants in this study were administered single doses of 150 mg ribavirin IV and 400 mg ribavirin PO. Blood samples were collected immediately prior to dosing and

then 0.083, 0.167, 0.333, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 7, 9, 11, 13, 17, 25, 37, 49, 61, 73, 97, 121, 145 and 169 hours after dosing.

Concentration-time data was extracted using Engauge Digitizer software (http://markummitchell.github.io/engauge-digitizer).

POPULATION PHARMACOKINETIC MODELLING

Model Development

Population pharmacokinetic modelling and simulation was conducted using NONMEM® VII (ICON Development Solutions, Ellicott City, MD, USA) software with an Intel Fortran complier and Wings for NONMEM interface (http://wfn.sourceforge.net).

One-, two- and three-compartment models with first-order absorption and elimination from the central compartment were fitted to plasma ribavirin concentration-time data. Models with absorption lag time were screened to account for any time delay between administration and the beginning of drug absorption. In addition, more complex absorption models based on a chain of absorption transit compartments were also investigated. The pharmacokinetic models were parameterised (where appropriate) as clearance (CL), volume of distribution of the central compartment (Vc), inter-compartmental clearance(s) (CLd), volume of distribution of the peripheral compartment(s) (Vp), absorption rate constant (Ka), absorption lag time (Alag) and bioavailability (F). The model incorporated population parameter variability (comprising between-subject and between-occasion variability) and residual unexplained variability (comprising proportional and/or additive error).

Once the base structural model had been determined, the contributions of continuous (age, body weight, renal function) and categorical (gender, patient type) covariates to population parameter variability were assessed using a forward selection – backward elimination procedure.

Model selection was based on the objective function value (minus twice the log-likelihood of the data) as well as visual inspection of the standard diagnostic plots. A statistically significant ($p<0.05$) improvement in the comparison of

nested models was defined as a decrease in the objective function value of 3.84 U (for 1 degree of freedom). The final population pharmacokinetic model was evaluated through visual predictive checks.

The population pharmacokinetic model was developed and evaluated in consultation with Dr Reuter Lange. Full details of the model development are contained within Appendix 1.

Monte Carlo Simulation

To assess the ability of current and alternate dosing regimens to meet target ribavirin concentrations over a 14-day treatment course, the final population pharmacokinetic model was used to simulate datasets for a representative patient population of 10,000 individuals. Model simulation was conducted using R® Version 3.3.2 (R Foundation for Statistical Computing).

CHANGES TO STUDY PROTOCOL

At the St Vincent's Hospital study site, rigorous adherence to the sampling protocol proved logistically impossible for several reasons including staff resources and patient preferences about sample collection timing. Additionally, sample collection often had to be negotiated around other clinical commitments for study investigators. Many patients expressed that the burden of clinic visits is heavy for transplant recipients and they would prefer not to present to the hospital for a blood-collection for the benefit of research alone. In response to this logistical difficulty a policy of sampling as intensely as possible within the available timeframe was adopted. This allowed for a rich data set, even if it were not possible to adhere to the original specified study protocol time-points.

CHAPTER 3: Results

STUDY POPULATION

A total of 120 plasma ribavirin concentrations from 24 lung transplant recipients, as well as 188 concentration-time data-points extracted from previously reported data for 6 healthy volunteers, were included in the population pharmacokinetic analysis. No data-points were excluded from the dataset. A summary of patient characteristics is included in Table 3.1.

Table 3.1: Participant Characteristics

Data expressed as mean ± standard deviation [range]

	St Vincent's Hospital Patients	**University Medical Centre Groningen Patients**	**Healthy Volunteers**
Count	11	13	6
Gender	7 Male / 4 Female	5 Male / 8 Female	6 Male / 0 Female
Age (years)	46.1 ± 14.3 [26 – 63]	53.2 ± 14.4 [27 – 73]	36.8 ± 5.34 [31 – 44]
Body Weight (kg)	65.6 ± 15.6 [47 – 105]	73.2 ± 18.4 [42 – 120]	78.3 ± 11.3 [58.7 – 89.9]
Creatinine Clearance (mL/min)	61.8 ± 30.7 [20.1 – 126]	56.6 ± 25.4 [25.1 – 111]	105 ± 13.2 [86.8 – 120
Time since Transplantation (years)	7.39 ± 4.85 [0.25 – 15.1]	4.06 ± 3.06 {0.25 – 7.00]*	N/A
Underlying Disease	4 Cystic Fibrosis / 4 COPD / 3 Alpha-1 Antitrypsin Deficiency / 1 Pulmonary Fibrosis / 1 Pulmonary Hypertension	2 Pulmonary Hypertension, 2 Cystic Fibrosis / 1 Interstitial Lung Disease / 1 COPD / 5 Unknown	N/A

* n = 4

No significant differences were found in participant characteristic data, with the exception of creatinine clearance which was significantly higher in healthy volunteers compared to the St Vincent's Hospital and University Medical Centre Groningen patient groups.

FINAL POPULATION PHARMACOKINETIC MODEL

A three-compartment model with first-order elimination from the central compartment was found to most adequately describe ribavirin concentration-

time data. The model incorporated population parameter variability for CL, Vc, CLd1, CLd2, Ka and F, and proportional residual unexplained variability.

Introduction of covariates into the structural model identified an effect of creatinine clearance (CrCL, calculated as Cockcroft-Gault equation) on CL, an effect of body weight (Wt) on Vc (incorporated using allometric scaling), and patient type (i.e. lung transplant recipient or healthy normal volunteer) on CL and Vc.

On model diagnostics, the model was found to well characterise the ribavirin concentration-time data and comparison of observed data and median and 90% prediction intervals of simulated data demonstrated close prediction over the time course of the study.

The final population pharmacokinetic model is illustrated in Figure 3.1 and population parameter estimates of the final pharmacokinetic model are presented in Table 3.2. A summary of model development and the final model code is included within Appendix 1.

Figure 3.1: Schematic of the final ribavirin population pharmacokinetic model

CL, clearance; Vc, volume of distribution of the central compartment; Vp, volume of distribution of the peripheral compartment; CLd, intercompartmental clearance; Ka, absorption rate constant; CrCL, creatinine clearnace; CrCL~CL, effect of creatinine clearance on clearance; Pt, patient type where 0 = healthy control and 1 = lung transplant recipient; Pt~CL, effect of patient type on clearance; PPV, population parameter variability.

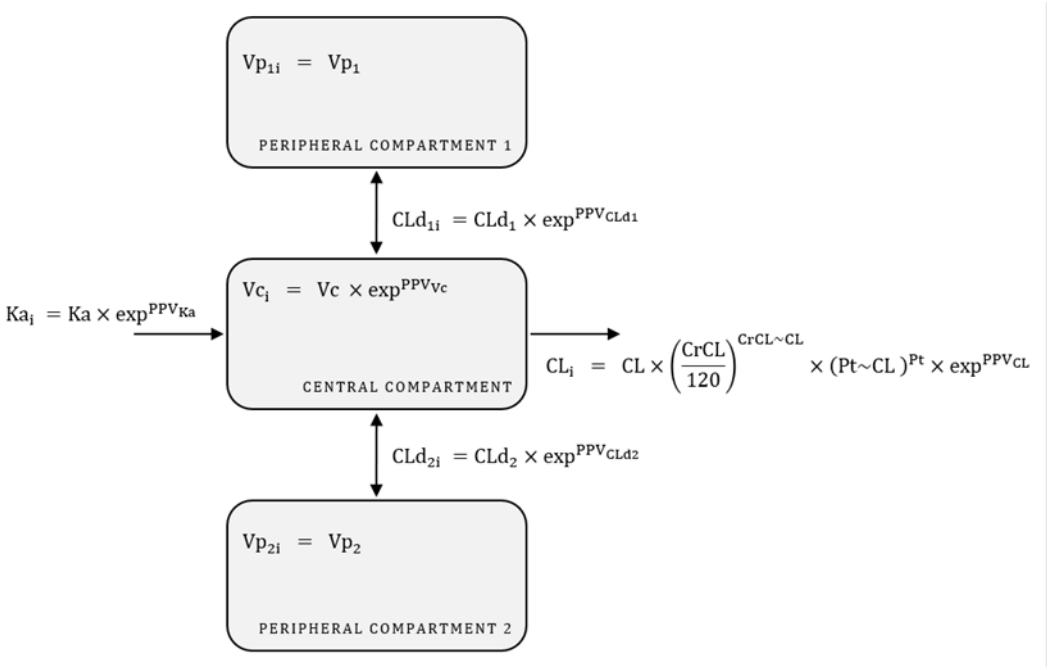


Table 3.2: Final Ribavirin Population Pharmacokinetic Model Parameter Estimates

CL, clearance; Vc, volume of distribution of the central compartment; Vp, volume of distribution of the peripheral compartment; CLd, intercompartmental clearance; Ka, absorption rate constant; F, oral bioavailability; CrCL~CL, effect of creatinine clearance on clearance; Pt~CL, effect of patient type on clearance; PPV, population parameter variability; %RSE, relative standard error, %CV, coefficient of variation.

Parameter	Final Model: Estimate (%RSE)	Shrinkage	Eta Bar P
Fixed Effects			
CL (L/hr)	17.5 (27.0%)		
Vc (L)	52.2 (18.6%)		
Vp1 (L)	152 (38.8%)		
Vp2 (L)	1140 (21.8%)		
CLd1 (L/hr)	40.7 (22.0%)		
CLd2 (L/hr)	39.8 (17.5%)		
Ka (hr-1)	0.318 (19.8%)		
F (%)	0.512 (12.1%)		
CrCL~CL	0.574 (64.6%)		
Pt~CL	0.586 (33.8%)		
Random Effects			
PPV CL (%CV)	34.9%	39.5%	0.759
PPV Vc (%CV)	34.1%	51.5%	0.803
PPV CLd1 (%CV)	34.6%	49.9%	0.787
PPV CLd2 (%CV)	49.4%	26.3%	0.842
PPV Ka (%CV)	19.7%	64.3%	0.946
PPV F (%CV)	44.8%	13.8%	0.938
Residual Variability			
Proportional (%CV)	25.7%	9.9%	

EXAMINATION OF DOSING REGIMENS

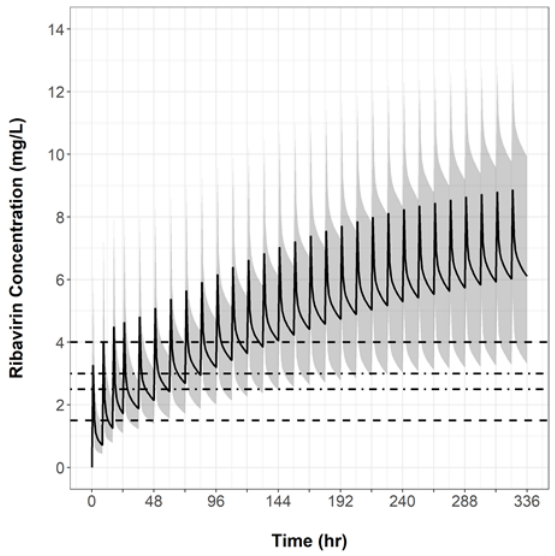
A representative patient population of 10,000 lung transplant recipients with distributions of creatinine clearance and associated body weights consistent with that seen within the patient cohort was constructed and concentration-time profiles simulated using the developed population pharmacokinetic model to examine the ability of the current and alternate dosing regimens to achieve target concentrations over a 14-day treatment course. It should be noted that for oral dosing regimens, doses administered were rounded to the nearest 200 mg (i.e. tablet size) so that recommended regimens could be administered to patients using currently available products (200mg tablets being the available preparation).

Based on the simulations, each of the standard dosing regimens currently used in practice (11 mg/kg TDS PO or IV + 10 mg/kg BD PO or IV) performed well to achieve target concentrations within the first 24 hours of dosing; however, despite the loading dose, steady-state concentrations were not achieved by the end of the 14-day treatment course (Figure 3.2). Plasma ribavirin concentrations continued to accumulate over the course of the treatment period such that >90% of patients are predicted to have concentrations well above the upper limit of the defined therapeutic range on Day 14. For the median patient, these concentrations were predicted to be 2- to 3-fold higher than the target concentrations. Similar results were observed when stratified by renal function, indicating that the observed drug accumulation was not primarily due to altered creatinine clearance in a patient subgroup.

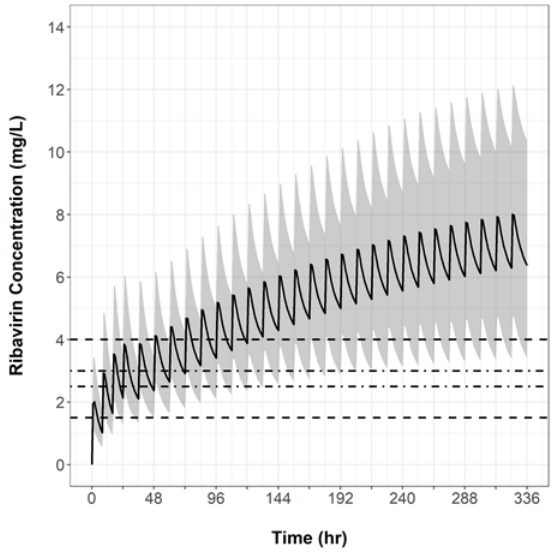
Figure 3.2: Plasma ribavirin concentration-time profile

Data presented as median solid line and 90% prediction intervals (shaded).

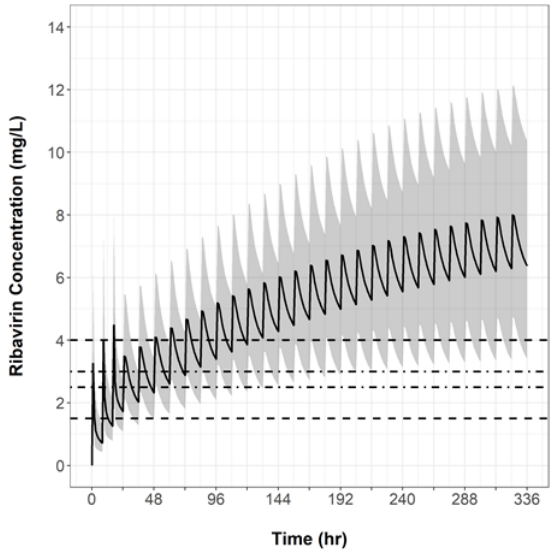
A. 11 mg/kg q8hIV for the first 24 hours, followed by a maintenance dose of 10 mg/kg q12h IV



B. 11 mg/kg q8h IV for the first 24 hours, followed by a maintenance dose of 10 mg/kg q12h PO



C. 11 mg/kg q8h PO for the first 24 hours, followed by a maintenance dose of 10 mg/kg q12h PO

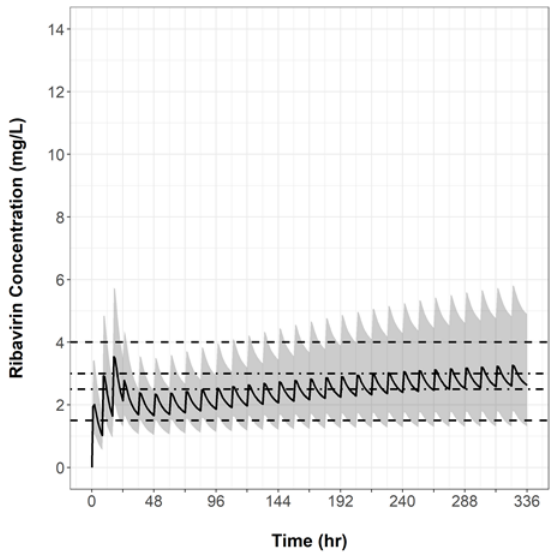


Alternate dosing regimens were simulated to determine doses predicted to result in concentrations within the therapeutic range at the end of the treatment period (Figure 3.3). An oral dosing regimen of 11 mg/kg TDS PO + 4 mg/kg BD PO (similar to current IV loading dose with ongoing dose reduced by half) was found to result in plasma ribavirin concentrations for the median patient between the desired 2.5 – 3 mg/L range, and >90% of patients achieving concentrations above 1.5 mg/L. However, despite the loading dose achieving therapeutic targets at the end of Day 1, plasma concentrations declined on Day 2 and then progressively increased by the end of the nominal treatment period. Consistent results were seen across the spectrum of renal functions. Similar results were seen for an all oral dosing regimen of 11 mg/kg TDS PO + 8 mg/kg d. PO.

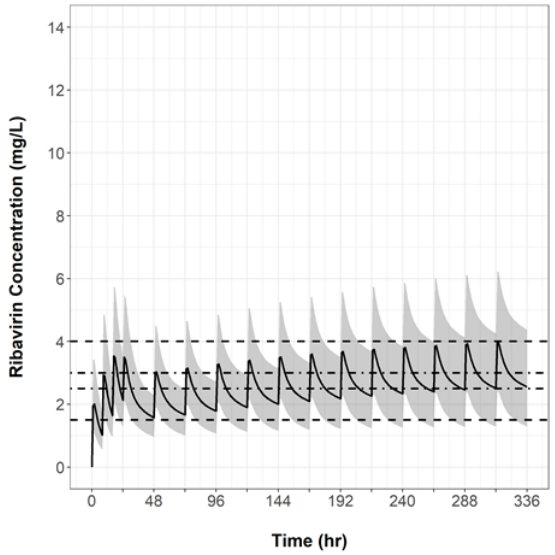
Figure 3.3: Plasma ribavirin concentration-time profile

Data presented as median solid line and 90% prediction intervals (shaded).

A. 11 mg/kg q8h PO for the first 24 hours, followed by a maintenance dose of 4 mg/kg q12h PO



B. 11 mg/kg q8h PO for the first 24 hours, followed by a maintenance dose of 8 mg/kg q24h PO

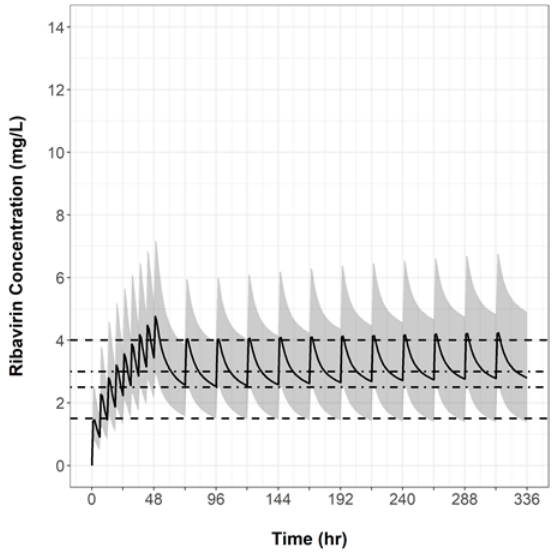


An alternate regimen was designed to achieve concentrations in the desired range at the beginning of treatment and maintain these throughout the treatment course (Figure 3.4). A regimen of 8 mg/kg QID PO for 48 hours followed by 8 mg/kg d. PO for the remainder of the treatment period was predicted to result in >90% of patients exhibiting concentrations within the defined target range throughout the entire treatment period. Slight drug accumulation is seen for patients with severe renal impairment (<30 mL/min); however, concentrations are predicted to remained below that seen for the dosing regimens currently used in clinical practice.

Figure 3.4: Plasma ribavirin concentration-time profile

Data presented as median solid line and 90% prediction intervals (shaded).

A. 8 mg/kg q6h PO for the first 48 hours, followed by a maintenance dose of 8 mg/kg q24h PO



CHAPTER 4: Discussion

Despite the use of ribavirin for the treatment of viral respiratory infections, little is known in regard to the ability of current dosing regimens to meet effective target plasma concentrations. This is particularly relevant for the lung transplant population for which sub-therapeutic treatment is likely to result in significant morbidity and reduced median survival as a result of BOS. Supra-therapeutic doses have been associated with haemolytic anaemia and renal impairment.[15] Evidence-based dosing regimens are therefore critical for the optimal treatment of these patients. This study undertook a population pharmacokinetic approach to determine the pharmacokinetic properties of ribavirin and the factors contributing to variability in this patient group. This then allowed for the examination of current and alternate dosing regimens and their ability to meet predefined therapeutic targets.

Our population pharmacokinetic analysis confirmed that ribavirin is characterised by three-compartmental pharmacokinetics, with first order elimination, consistent with compartmental analysis previously reported[8] and the known pharmacokinetic properties of ribavirin that have been previously described.[15] The model found total body clearance to be influenced by creatinine clearance (with a 10% reduction in CrCL associated with a 6% reduction in systemic clearance), this is not unexpected given that renal clearance comprises approximately one-third of total clearance. Patient type also influenced clearance, with lung transplant recipients exhibiting a 41% reduction in systemic clearance when compared with healthy controls.

Simulations of current IV and/or PO ribavirin dosing regimens indicate that, whilst administration of the standard loading doses result in plasma concentrations within the target therapeutic range early in the treatment course, concentrations continue to accumulate throughout the 14-day treatment period such that later concentrations are substantially higher than the defined upper limit of 4.0 mg/L.[46,47] Previous studies indicated that ribavirin concentrations >3.5 mg/L are associated with severe side effects, even after only a few days of treatment.[44-47] Therefore, it is feasible that these regimens may be associated with increased risk of adverse effects. On the other hand, alternate dosing regimens that target effective concentrations at the end of the treatment period are

predicted to result in sub-therapeutic concentrations during the first days of treatment, thereby potentially placing patients at risk of ineffective treatment. Utilising the developed pharmacokinetic model and known characteristics of ribavirin, this study has been able to propose a dosing regimen, consisting of 8 mg/kg q6h oral for the first 48 hours, followed by a maintenance dose of 8 mg/kg q24h oral, that is predicted to result in early attainment of therapeutic concentrations and continued maintenance at these levels throughout the treatment course. Arguably, this indicates that therapeutic levels can be achieved with a 45% reduction in total dose administered; the combination of oral administration and dose reduction has potential for substantial cost savings. Notably, whilst this regimen includes higher loading doses, the predicted exposure remains below that observed later in the treatment period for the current regimens and therefore is considered to pose no additional risk of toxicity.

STUDY STRENGTHS AND WEAKNESSES

Whilst this study provides valuable information on the pharmacokinetics of ribavirin in the lung transplant population, the majority of patients within this study were recruited as outpatients and no severe inflammatory states were seen. As such, the impact of this more complex clinical situation on ribavirin pharmacokinetics is not able to be determined from this analysis. Furthermore, due to limited data and/or lacking information for some patients, the influence of factors, such as underlying disease (including cystic fibrosis), time since transplantation, or immunosuppressive scheme, could not be determined. Preliminary examination of the data indicated no discernible trends; however, full exploration with a larger dataset would be required for definitive conclusions to be made.

Importantly, it should be noted that current evidence for the proposed therapeutic target concentrations utilised within this study is limited and requires additional work. Longitudinal research examining the concentration-adverse effect relationship would be desirable in order to provide more evidence of the upper limit of ribavirin concentrations that can be tolerated for short-term therapy and how this relates to what is currently considered “safe”.

Nonetheless, the developed population pharmacokinetic model provides an effective tool for anticipating ribavirin exposure in a population with unique antimicrobial needs. The use of this innovative methodology allows for the development and examination of optimal treatment regimens without the need for costly, large-scale clinical trials, providing an evidence basis for effective treatment protocols.

This study provides a valuable first step in determining optimal ribavirin treatment regimens for paramyxovirus infections in the lung transplant population. Results of this work suggest that a regimen of 8 mg/kg QID PO for 48 hours followed by 8 mg/kg daily PO for the remainder of the treatment period is effective in maintain >90% of patients within the currently defined target range for the duration of treatment.

CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS

Once the target therapeutic range for ribavirin is formally established, this study will provide a foundation for ascertaining the safest and most effective dosing regimen to achieve target plasma concentrations throughout the dosing interval.

There is a growing body of evidence that plasma concentrations display heterogeneity between patients, even with identical dosing regimens. A guideline-recommended course of antimicrobial therapy may result in toxicity in one patient whilst failing to reach effective concentrations in another. Guidelines for the "general population" thus do not apply to all patients. Studies such as the one we have conducted provide important information for generating appropriate and personalised antimicrobial doses for specific populations and specific patients. In the future, this research has the potential to provide the basis for a new era of individualised treatment, with other key pillars under development being individual genetic and metabolic analysis. This evolution of treatment has the potential to result in more effective regimens with reduced mortality, minimisation of the development of antimicrobial resistance and reduced drug toxicity. It is imperative that more research is done to identify effective therapeutic concentrations and safety profiles for optimal treatment and patient outcomes.

CHAPTER 5: Personal Reflection

There were many setbacks in the execution of this project which led to a delay in the final submission of our findings to the Journal of Antimicrobial Chemotherapy. As a developing investigator overcoming these challenges proved a valuable learning experience in the completion of this Masters project. Early in the development of the study protocol, funding earmarked from a hospital trust to provide for a dedicated venepuncture assistant to collect plasma samples were suddenly unavailable. This led to myself, as the study investigator, undertaking most of the data collection with some help from volunteers. Project coordination was thus a greater than expected workload. Ultimately, this setback had the positive impact of affording the opportunity to develop skills in complex study coordination with direct involvement in every step of the process from patient consent to blood collection, sample processing, data analysis and finally authoring a manuscript for publication. The experience of juggling a research degree whilst undertaking full-time medical training program, whilst challenging, was a good way to develop skills in time management and productivity.

The flexibility of the investigating team proved useful again in mid 2014 when a manufacturing issue interrupted the supply chain of IV ribavirin to Australia. Oral stock remained unaffected so patients were given substituted oral loading doses at the discretion of their treating clinicians. Instead of giving-up on the work already done, we continued the project and the data collected during this incident afforded greater opportunity to compare mixed IV/PO versus PO only regimes. Eventually supply was re-established and the project continued as per the original study protocol.

Several of the team members working on the initial data collection phase had personal difficulties during the completion of this project. Unfortunately,

interpersonal conflict between the other team members also developed. These incidents became valuable exercises in developing mediation skills to use when supervising junior staff. Under the guidance of Professor Richard Day (the head of the St Vincent's Department of Clinical Pharmacology) we led mediation sessions in which we developed strategies for individuals in conflict to continue working together. Moving a project forward through differing expectations, workloads and standards of professional behaviour was an ongoing challenge and, I imagine, something that affects all research projects. Nevertheless, developing the skill of personnel management is an asset to any study coordinator.

To add an extra challenge but also advantage to our study, project supervisor Professor Deborah Marriott, developed a partnership with the University Medical Centre Groningen Transplant Unit in the Netherlands after the initial data collection phase had begun. Favourably, this professional relationship doubled our patient cohort. Although of great benefit to the project, this did add a layer of difficulty due to the demands of coordinating data collection and analysis between two international centres. Although not easy, collaborating over great distances is again a useful experience for clinicians as multi-centre studies are usually of a higher standard and achieve statistical significance more reliably that single-centre cohorts.

APPENDIX 1: Population Pharmacokinetic Model

MODEL DEVELOPMENT

Pharmacokinetic parameters were estimated using non-linear mixed effects modelling using first-order conditional estimation (FOCE) with interaction. The population parameter variability (comprising between-subject variability and between-occasion variability) was modelled using an exponential random effect model:

$\theta_i = \theta \cdot \exp(\eta_i)$

where: θ_i is the pharmacokinetic parameter for the i^{th} individual; θ is the population pharmacokinetic parameter; η_i is a random variable of population parameter variability which is assumed to be normally distributed around zero with a variance of ω^2 to distinguish the i^{th} patients value from the population estimate. The population parameter variability was expressed as a coefficient of variation (%CV, approximated by the square root of the variance estimate).

The residual unexplained variability, arising from such factors as experimental errors and model misspecification, was modelled with the use of a combined proportional and additive model:

$C_{ij} = \hat{C}_{ij} \cdot (1 + \varepsilon_{1,ij}) + \varepsilon_{2,ij}$

where: C_{ij} is the j^{th} observed concentration for the i^{th} individual; $\hat{C}_{ij}$ is the corresponding predicted concentration; $\varepsilon_{1,ij}$ and $\varepsilon_{2,ij}$ are randomly distributed variables with a mean of zero and variances of σ_1^2 (expressed as %CV) and σ_2^2 (expressed as standard deviation), respectively.

Once the base structural model had been determined, linear regression was used to screen for associations between each of the individual post-hoc parameters and available covariates. Various models were then examined

incorporating effects for covariates identified from linear regression screening. Continuous covariates were centred on typical values such that population estimates represent that for an average patient and were incorporated into the structural model as per that for body weight:

$\theta_i = [\theta \, . \, Weight/70) \, \hat{} \, \theta_2] \, . \, \exp(\eta_i)$

where: θ_i is the pharmacokinetic parameter for the i^{th} individual; θ is the population pharmacokinetic parameter; θ_2 is the effect of the covariate; η_i is the random effect of population parameter variability.

Categorical covariates were introduced into the structural model as per that for gender:

$\theta_i = [\theta \, . \, \theta_2 \, \hat{} \, Gender)] \, . \, \exp(\eta_i)$

where: θ_i is the pharmacokinetic parameter for the i^{th} individual; θ is the population pharmacokinetic parameter; θ_2 is the effect of the covariate; η_i is the random effect of population parameter variability.

After incorporation of all significant covariates into the model, a backward elimination process was employed to confirm the relevance of each covariate in the final model.

MODEL DIAGNOSTICS

The adequacy with which the pharmacokinetic model described the concentration-time data was determined through examination of the precision of parameter estimates and objective function value. A reduction in the objective function value by 3.84 for the addition of a single parameter was considered statistically significant ($p<0.05$) and representative of improvement. In conjunction with the objective function value, alternative selection measures, including Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) were also considered. A reduction in the AIC and/or BIC from

the initial model to the subsequent model were confirmation of improvement. The AIC and BIC were defined by the following equations:

$AIC = OFV + 2p$

$BIC = OFV + (p \cdot \log N)$

where: OFV is the objective function value; p is the number of unknown parameters (θ, σ^2 and ω^2) in the model; N is the number of observations.

Model selection was also based on visual inspection of standard diagnostic plots including observed concentrations versus population predicted and individual predicted concentrations, and conditional weighted residuals versus population predicted concentrations and time.

To assess the reliability of the post-hoc individual parameter estimates for model diagnostics and comparison, the shrinkage of the empirical Bayes estimates was calculated. In addition, to test the hypothesis that the post-hoc individual parameters are centred on the population estimate, eta-bar p values were calculated.

A visual predictive check was used to characterise the performance of the final model. The parameter estimates, population parameter variability and residual unexplained variability were used to generate 1000 simulated datasets from which median and 90% prediction intervals (5^{th} and 95^{th} percentiles) of the simulated values were determined and compared to observed values.

FINAL POPULATION MODEL

Model development is outlined in Table A1.1. The effects of covariates on random effects are presented in Figure A1.1.

The diagnostic plot of individual predicted concentrations versus observed concentrations was symmetrically distributed around the line of unity and no

trends were observed in the conditional weighted residuals diagnostic plots, indicating that the model adequately describes the ribavirin pharmacokinetic profile (Figure A1.2).

The control stream for the final population pharmacokinetic model is presented in Appendix A1.1.

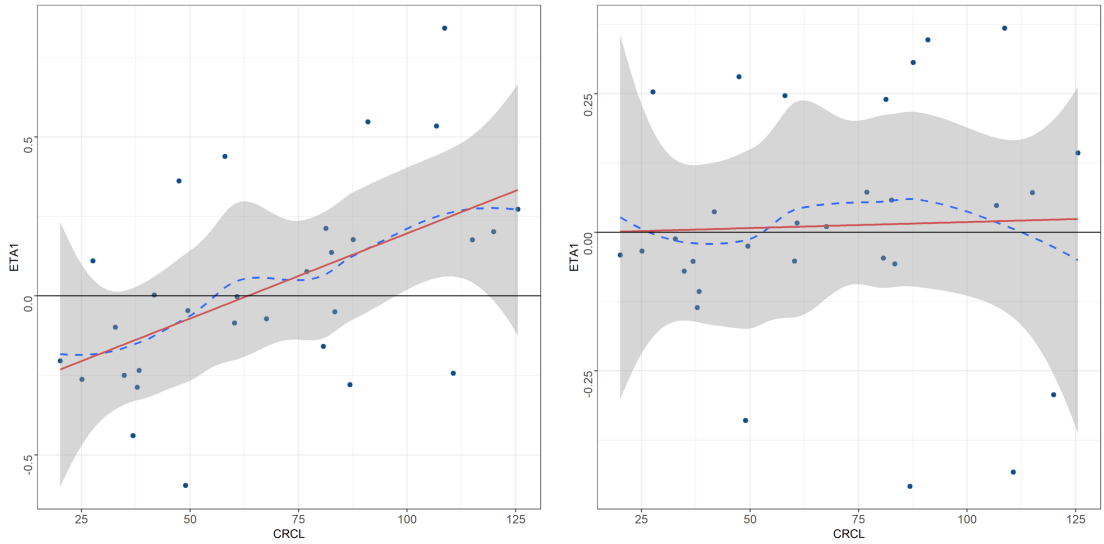
Table A1.1: Summary of Model Development

PPV, population parameter variability; Vp, volume of distribution of the peripheral compartment; CrCL~CL, effect of creatinine clearance on clearance; Wt~Vc, effect of weight on central volume of distribution; Pt~CL, effect of patient type on clearance; Pt~Vc, effect of patient type on central volume of distribution; ΔOFV, change in objective function value; ΔAIC, change in Akaike Information Criterion; ΔBIC, change in Bayesian Information Criterion.

Model ID	Model Description	Model Comparison
Structural Model Development		
RBVPopPKModel01	1 compartment	
RBVPopPKModel02	2 compartment	c.f. RBVPopPKModel01 ΔOFV: -373.96 ΔAIC: -365.27 ΔBIC: -350.35
RBVPopPKModel03	3 compartment	c.f. RBVPopPKModel02 ΔOFV: -42.97 ΔAIC: -34.04 ΔBIC: -19.12
RBVPopPKModel04	RBVPopPKModel03 + Transit	c.f. RBVPopPKModel03 ΔOFV: 17.21 ΔAIC: 17.21 ΔBIC: 17.21
RBVPopPKModel05	RBVPopPKModel04 – Additive Error	c.f. RBVPopPKModel03 ΔOFV: -0.75 ΔAIC: -3.01 ΔBIC: -6.74
RBVPopPKModel06	RBVPopPKModel05 – PPVVp1	c.f. RBVPopPKModel05 ΔOFV: 0.38 ΔAIC: -1.86 ΔBIC: -5.59
RBVPopPKModel07	RBVPopPKModel06 – PPVVp2	c.f. RBVPopPKModel06 ΔOFV: 0 ΔAIC: -2.23 ΔBIC: -5.96
RBVPopPKModel08	RBVPopPKModel07 + Covariance Matrix	c.f. RBVPopPKModel07 ΔOFV: -38.22 ΔAIC: -3.15 ΔBIC: 52.8
Full (Covariate) Model Development		
RBVPopPKModel09	RBVPopPKModel07 + CrCL~CL	c.f. RBVPopPKModel07 ΔOFV: -8.98 ΔAIC: -6.75 ΔBIC: -3.02
RBVPopPKModel10	RBVPopPKModel09 + Wt~V2	c.f. RBVPopPKModel09 ΔOFV: -0.03 ΔAIC: 2.21 ΔBIC: 5.94
RBVPopPKModel11	**RBVPopPKModel09 + Pt~CL**	**c.f. RBVPopPKModel09** **ΔOFV: -2.92 ΔAIC: -0.68 ΔBIC: 3.05**
RBVPopPKModel12	RBVPopPKModelPK11 + Pt~V2	c.f. RBVPopPKModel11 ΔOFV: -1.98 ΔAIC: 0.28 ΔBIC: 4.01
RBVPopPKModel13	RBVPopPKModel11 – CRCL~CL	c.f. RBVPopPKModel11 ΔOFV: 3.48 ΔAIC: 1.24 ΔBIC: -2.49

Figure A1.1: Effect of the Introduction of Covariates on Random Effects (ETA).

A. *Random effect for clearance (CL) versus creatinine clearance (CrCL) obtained from the base structural model and after the introduction of CrCL as a covariate on CL.*



B. *Random effect for clearance (CL) versus patient type (Pt) obtained from the base structural model and after the introduction of Pt as a covariate on CL.*

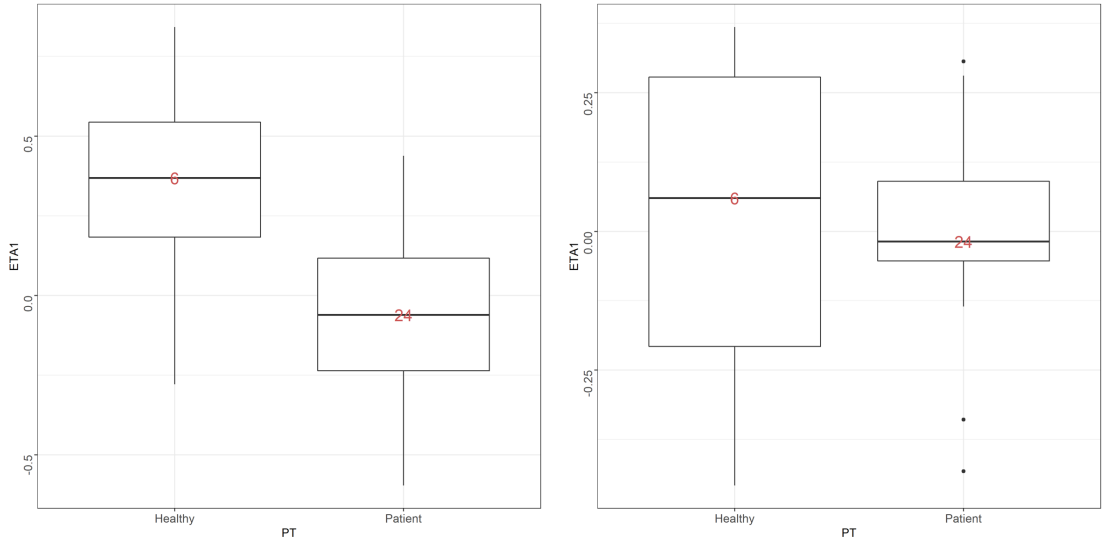
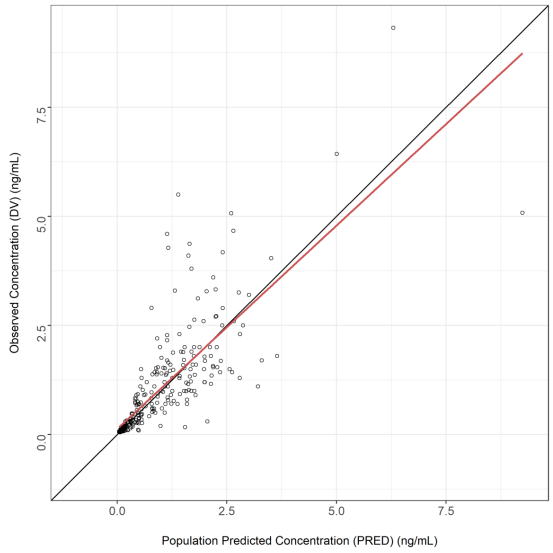
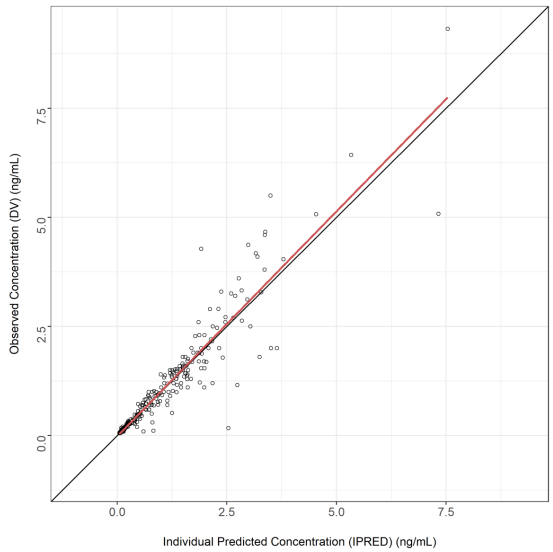


Figure A1.2: Final Population Pharmacokinetic Model Diagnostic Plots.

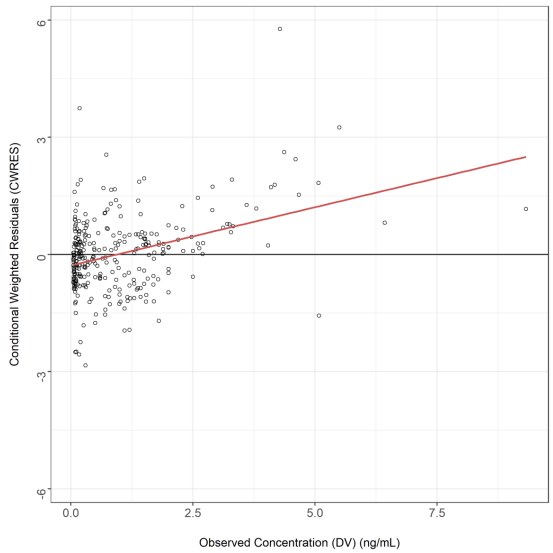
A. *Population predicted concentration versus observed concentration, including line of unity (black line) and line of best fit (red line).*



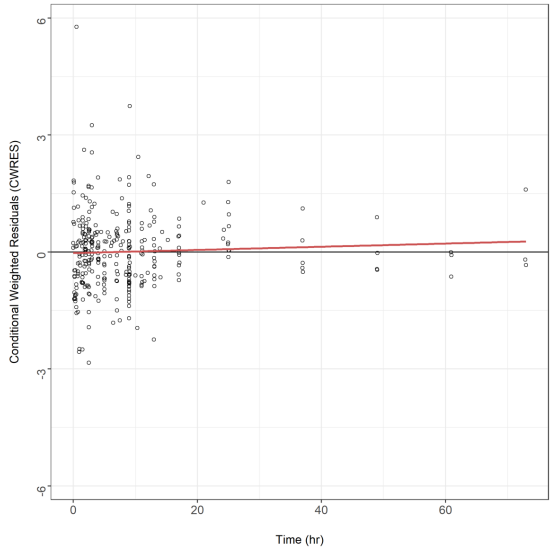
B. *Individual predicted concentration versus observed concentration, including line of unity (black line) and line of best fit (red line).*



C. *Conditional weighted residuals versus observed concentration, including zero line (black line) and line of best fit (red line).*



D. *Conditional weighted residuals versus time, including zero line (black line) and line of best fit (red line).*



Appendix A1.1 – Model Code

```
$PROBLEM        Ribavirin PK in Lung Transplant

$DATA           Data.csv

$INPUT          ID TIME AMT RATE DV MDV EVID CMT PT SEX AGE WT HT SCR IBW CRCL

$SUBROUTINES    ADVAN6 TOL=9

$MODEL          NCOMPARTMENTS=4
                COMP=(DEPOT)
                COMP=(CENTRAL,DEFOBS)
                COMP=(PERIPH1)
                COMP=(PERIPH2)

$PK             POPCL   =  THETA(1)
                POPV2   =  THETA(2)
                POPV3   =  THETA(3)
                POPV4   =  THETA(4)
                POPQ1   =  THETA(5)
                POPQ2   =  THETA(6)
                POPKA   =  THETA(7)
                POPF1   =  THETA(8)
                CRCL_CL =  THETA(9)
                PT_CL   =  THETA(10)

                PPVCL   =  ETA(1)
                PPVV2   =  ETA(2)
                PPVQ1   =  ETA(3)
                PPVQ2   =  ETA(4)
                PPVKA   =  ETA(5)
                PPVF1   =  ETA(6)

                CL      =  POPCL*((CRCL/120)**CRCL_CL)*(PT_CL**PT)*EXP(PPVCL)
                V2      =  POPV2*EXP(PPVV2)
                V3      =  POPV3
                V4      =  POPV4
                Q1      =  POPQ1*EXP(PPVQ1)
                Q2      =  POPQ2*EXP(PPVQ2)
                KA      =  POPKA*EXP(PPVKA)
                F1      =  POPF1*EXP(PPVF1)

                S2      =  V2
```

```
$DES            C2      =  A(2)/V2
                C3      =  A(3)/V3
                C4      =  A(4)/V4

                DADT(1) = -A(1)*KA
                DADT(2) =  A(1)*KA -C2*Q1 + C3*Q1 -C2*Q2 + C4*Q2 -C2*CL
                DADT(3) =           C2*Q1 - C3*Q1
                DADT(4) =                           C2*Q2 - C4*Q2


$THETA          (0,17.5,)       ;POPCL
                (0,52.2,)       ;POPV2
                (0,152,)        ;POPV3
                (0,1140,)       ;POPV4
                (0,40.7,)       ;POPQ1
                (0,39.8,)       ;POPQ2
                (0,0.318,)      ;POPKA
                (0,0.512,1)     ;POPF1
                (-INF,0.574,)   ;CRCL_CL
                (0,0.586,)      ;PT_CL


$OMEGA          0.1218          ;PPVCL
                0.1163          ;PPVV2
                0.1197          ;PPVQ1
                0.2440          ;PPVQ2
                0.0388          ;PPVKA
                0.2007          ;PPVF1


$SIGMA          0.0660          ;ERRPROP


$ERROR          Y  =  F*(1+ERR(1))
                IPRED  =  F


$ESTIMATION     METHOD=1  INTERACTION  MAXEVALS=9999 POSTHOC  NOABORT
                NSIG=3 SIGL=9

$COVARIANCE     UNCONDITIONAL SLOW SIGL=12 PRINT=E

$TABLE          ID TIME AMT RATE CL V2 V3 V4 Q1 Q2 KA F1 ETA1 ETA2 ETA3 ETA4
                ETA5 ETA6 IPRED CWRES PT SEX AGE WT HT SCR IBW CRCL NOPRINT
                ONEHEADER FILE=
```

APPENDIX 2: Publications

MANUSCRIPT

Milliken E, de Zwart AES, Alffenaar JC, Marriott DJE, Riezebos-Brilman A, Schteinman A, Evans AM, Glanville AR, Verschuuren EAM, Reuter SE. Population pharmacokinetics of ribavirin in lung transplant recipients and examination of current and alternative dosing regimens. J Antimicrob Chemother; DOI 10.1093/jac/dky466 [Epub ahead of print]

J Antimicrob Chemother
doi:10.1093/jac/dky466

Journal of Antimicrobial Chemotherapy

Population pharmacokinetics of ribavirin in lung transplant recipients and examination of current and alternative dosing regimens

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Background: Ribavirin is used in the treatment of respiratory paramyxovirus infection in lung transplant recipients; however, its pharmacokinetic profile in the transplant population is unknown despite the potential for alterations due to underlying pathology. Furthermore, the ability of current regimens to meet exposure targets has not been established.

Objectives: This study examined the pharmacokinetics of ribavirin in a lung transplant population for which current and alternative dosing regimens were assessed.

Methods: Population pharmacokinetic modelling was conducted in NONMEM using concentration–time data from 241 lung transplant recipients and 6 healthy volunteers. Monte Carlo simulation was used to assess the ability of dosing regimens to achieve pre-specified target concentrations.

Results and conclusions: A three-compartment model with first-order elimination most adequately described ribavirin concentration–time data, with $Cl_{r/f}$ and patient type (i.e. lung transplant) identified as significant covariates in the model. Simulations indicate that current regimens achieve efficacious concentrations within 24 h of treatment initiation that increase to supra-therapeutic levels over the treatment period. A regimen of 8 mg/kg q8h orally for 48 h followed by 8 mg/kg q24h orally for the remainder of the treatment period was predicted to result in >80% of patients exhibiting concentrations within the defined target range throughout the entire treatment course. Additional work to formally establish target therapeutic concentrations is required; however, this study provides a valuable first step in determining optimal ribavirin treatment regimens for paramyxovirus infections in the lung transplant population.

Introduction

Ribavirin is a guanosine analogue that is used in the treatment of paramyxovirus infections.[1] Historically, its primary respiratory indication has been treatment of respiratory syncytial virus (RSV) pneumonitis in paediatric and haematological malignancy or stem cell transplant populations.[2–5] More recently its use has been extrapolated to pulmonary transplant patients and the treatment of other paramyxoviruses, including human metapneumovirus (HMPV) and parainfluenza virus.[6–7] Goals of treatment are to reduce morbidity and mortality from acute pneumonitis as well as reduce the incidence of the bronchiolitis obliterans syndrome or transplant-associated pathological airway destruction, which has been linked to infection with respiratory viruses.[8,9] Both acute infection and bronchiolitis obliterans syndrome are major contributors to reduction in median survival post-thoracic transplantation

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and improved antimicrobial strategies are paramount to mortality reduction.[5,7,8] With the advent of new direct-acting antiviral agents for hepatitis C, the treatment of paramyxoviruses has taken over as a primary use for ribavirin. While ribavirin is available in aerosolized, intravenous and oral formulations, previous studies have focused mainly on the efficacy of aerosolized ribavirin, while only a single study has directly compared two types of formulations for paramyxovirus infections.[10,11] Despite this, most international thoracic transplant centres have opted for less expensive and less cumbersome oral and intravenous preparations, which may have equal efficacy.[12–15] Although ribavirin is in common use, its pharmacokinetics in the unique transplant population are not well understood. It is feasible that transplant recipients as a population may manifest deviations in the pharmacokinetic profile of antimicrobials and other drugs due to physiological changes induced by the transplant. Such changes include a high incidence of renal failure, polypharmacy (immunosuppressive regimens) and heavy corticosteroid exposure with associated alterations in hepatic metabolism. In cystic fibrosis patients, drug pharmacokinetics may be altered by abnormal gastrointestinal absorption; however, as total body fat and stomach contents influence the absorption and distribution of ribavirin, this may be an issue even in non-cystic fibrosis transplant recipients as the prevalence of malnutrition is high in this population.

Ribavirin is characterized by multi-compartmental pharmacokinetics with distribution of the drug into tissues and a characteristic long terminal half-life.[16] The antiviral effect of ribavirin may be dependent on dosage and the resulting plasma concentrations of the drug; thus, its pharmacokinetic properties are critical to the identification of optimal treatment protocols.[17–21] However, the evidence for a target effective plasma concentration is lacking, although the IC_{50} for RSV replication has been estimated at 1.35–5.82 mg/L *in vitro*, and values for HMPV may be similar to those for RSV.[18–20] Previous research has indicated that effective plasma concentrations are >2.5 mg/L; however, given the known association between plasma concentrations >3.5 mg/L and haemolytic anaemia,[21–24] it is considered reasonable to propose a target therapeutic range of 2.5–3.0 mg/L, with outer limits of 1.5–4.0 mg/L.

Ribavirin dose regimens for pulmonary virus infections have been derived from hepatitis C treatment. In order to achieve early steady-state concentrations, treatment strategies for oral or intravenous administration typically comprise loading doses of 11 mg/kg q8h for the first 24 h of treatment, followed by a maintenance dose of 10 mg/kg q12h. Despite established clinical protocols, target attainment of these regimens has not been previously examined in lung transplant recipients, who may have altered pharmacokinetics due to changes in underlying physiology. In order to investigate this further, a population pharmacokinetic approach was used to examine the pharmacokinetic properties, and sources of variability, of ribavirin in lung transplant recipients and healthy normal volunteers. Utilizing this model, the ability of current and alternative dosing regimens to meet target concentrations was examined.

Patients and methods

Pharmacokinetic data were prospectively and retrospectively obtained from lung transplant recipients diagnosed with a paramyxovirus infection recruited from study centres at St Vincent's Hospital (Sydney, Australia) and University Medical Centre Groningen (The Netherlands). Additional concentration–time data were extracted using Engauge Digitizer software (http://markummitchell.github.io/engauge-digitizer) from previously published work examining the pharmacokinetics of oral and intravenous ribavirin in healthy volunteers.[25]

The study was reviewed and approved by the Institutional Human Research Ethics Committees (St Vincent's Hospital Sydney Human Research Ethics Committee HREC/13/SVHH/74 and Medisch Ethische Toetsingscommissie University Medical Centre Groningen METc 2013/543). Prospectively examined participants were fully informed of the study procedures and provided written informed consent prior to study initiation. For retrospective participants, given the nature of the study, the need for written informed consent was waived by the local ethics committee. The study was conducted in accordance with the study protocol, the Declaration of Helsinki and Good Clinical Practice guidelines.

To be deemed eligible for study inclusion, lung transplant recipients were required to be >18 years of age and receiving ribavirin treatment for respiratory paramyxovirus infection. Patients receiving haemodialysis treatment were excluded from participation. No formal restrictions on time since transplantation were included as part of the eligibility criteria, but all patients were required to be stable since receipt of their transplant and not to have been transplanted for a minimum of 6 months.

Study procedures

Patients were initiated on oral and/or intravenous ribavirin treatment according to local standard treatment protocols and blood samples for analysis of plasma ribavirin concentrations were collected at various times after dose administration.

St Vincent's Hospital protocol

Study participants were administered a mixed intravenous/oral ribavirin dosing regimen consisting of a loading dose of 11 mg/kg q8h intravenously as a 30 min infusion for the first 24 h, followed by a maintenance dose of 10 mg/kg q12h orally. Blood samples were collected immediately prior to the first dose, immediately after completion of the infusion and then 1, 2, 3, 4, 6 and 8 h after the start of the first infusion. Additional samples were also collected on the morning of days 4 and 7 immediately prior to dosing and then 2 h after dose administration.

University Medical Centre Groningen protocol

Patients were treated with oral ribavirin administered as a loading dose of 11 mg/kg q8h for the first 24 h, followed by a maintenance dose of 10 mg/kg q12h. As part of standard care, blood samples were collected for monitoring of plasma ribavirin concentrations at 1.5–4 h after administration of the first dose and then immediately prior to morning dose administration on days 2, 4, 7 and 10.

Healthy normal volunteer dataset

Ribavirin pharmacokinetics were examined in healthy normal volunteers, as previously described.[25] In brief, participants were administered single doses of 1 50 mg of $^{13}C_2$-ribavirin intravenously and 400 mg of ribavirin orally. Blood samples were collected immediately prior to dosing and then 0.083, 0.167, 0.3 33, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 7, 9, 11, 13, 17, 25, 37, 49, 65, 73, 97, 121, 145 and 169 h after dose administration.

Biological sample analysis

Blood samples were processed immediately after collection and stored at −20°C until analysis. Plasma samples were analysed for ribavirin concentrations by the Department of Clinical Pharmacology and Toxicology of St Vincent's Hospital and the Department of Clinical Pharmacy and Pharmacology of University Medical Centre Groningen using validated LC

Table 1. Participant characteristics

	St Vincent's Hospital patients	University Medical Centre Groningen patients	Healthy volunteers
Count	11	13	6
Gender	7 male/4 female	5 male/8 female	6 male/0 female
Age (years), mean ± SD (range)	46.3 ± 14.3 (26–61)	53.2 ± 16.4 (27–71)	36.8 ± 5.36 (31–44)
Body weight (kg), mean ± SD (range)	65.6 ± 15.6 (47–105)	73.2 ± 18.6 (62–120)	78.3 ± 13.3 (58.7–89.9)
CL_{CR} (mL/min), mean ± SD (range)	61.8 ± 30.7 (20.1–126)	56.6 ± 25.6 (25.3–111)	105 ± 13.2 (86.8–120)
Time since transplantation (years), mean ± SD (range)	7.39 ± 4.85 (0.25–15.1)	4.06 ± 3.06 (0.25–7.00)[a]	NA
Underlying disease	2 pulmonary hypotension/2 cystic fibrosis/1 interstitial lung disease/1 COPD/5 unknown	4 cystic fibrosis/4 COPD/1 anti-trypsin deficiency/1 pulmonary fibrosis/1 pulmonary hypotension	NA

No significant differences were found in participant characteristic data, with the exception of CL_{CR} which was significantly higher in healthy volunteers as compared with the St Vincent's Hospital and University Medical Centre Groningen patient groups.
[a]$n = 4$.

random MS methodology.[20] Values for samples returning concentrations below the lower limit of quantification of the assay (0.2 mg/L) were not made available by the analytical laboratory; these samples were excluded from the pharmacokinetic dataset.

Population pharmacokinetics

Population pharmacokinetic modelling and simulation were conducted using NONMEM® VII (ICON Development Solutions, Ellicott City, MD, USA) software with an Intel Fortran compiler and Wings for NONMEM interface (http://wfn.sourceforge.net). Full details of the model development protocol and model diagnostics are available as Supplementary data at *JAC* Online; the pharmacokinetic methods are briefly described below.

One-, two- and three-compartment models with first-order absorption and elimination from the central compartment were fitted to plasma ribavirin concentration–time data. Models with more complex absorption characteristics based on a chain of absorption transit compartments were also investigated. The pharmacokinetic models were parameterized (where appropriate) as CL, volume of distribution of the central compartment (Vc), intercompartmental clearance(s) (CLd), volume of distribution of the peripheral compartment(s) (Vp), absorption rate constant (Ka) and bioavailability (F). The model incorporated population parameter variability (comprising between-subject and between-occasion variability) and residual unexplained variability (comprising proportional and/or additive error).

Once the base structural model had been determined, the contributions of continuous (e.g. age, body weight, renal function) and categorical (e.g. gender, patient type) covariates to population parameter variability were assessed using a forward selection–backward elimination procedure.

Model selection was based on the objective function value (minus twice the log-likelihood of the data) as well as visual inspection of the standard diagnostic plots. A statistically significant ($P < 0.05$) improvement in the comparison of nested models was defined as a decrease in the objective function value of 3.84 U (for 1 degree of freedom). The final population pharmacokinetic model was evaluated through visual predictive checks.

Monte Carlo simulation

To assess the ability of current and alternative dosing regimens to meet target ribavirin concentrations over a 14 day treatment course, the final population pharmacokinetic model was used to simulate datasets for a representative patient population of 10 000 individuals. Model simulation was conducted using R® Version 3.3.2 (R Foundation for Statistical Computing). Dosing regimens were assessed on the ability to meet the pre-specified target therapeutic range (1.5–4.0 mg/L) over the dosing interval; target attainment for 90% of patients was considered clinically relevant.

Results

A total of 120 plasma ribavirin concentrations from 24 lung transplant recipients ($n = 11$ at St Vincent's Hospital, $n = 13$ at University Medical Centre Groningen) and 188 concentration–time data points extracted from previously reported data for 6 healthy normal volunteers[21] were included in the population pharmacokinetic analysis. No data points were excluded from the dataset. A summary of patient characteristics is presented in Table 1.

Population pharmacokinetic model

A three-compartment model with first-order elimination from the central compartment was found to describe ribavirin concentration–time data most adequately. The model incorporated population parameter variability for CL, Vc, $CLd1$, $CLd2$, Ka and F, and proportional residual unexplained variability. The final population pharmacokinetic model is illustrated in Figure 1 and population parameter estimates of the final pharmacokinetic model are presented in Table 2. A summary of model development is presented in Table S1 and the final model control stream is included in Appendix S1.

Introduction of covariates into the structural model identified an effect of CL_{CR} (calculated using the Cockcroft–Gault equation) and patient type (i.e. lung transplant recipient or healthy normal volunteer) on CL (Figure S1).

Based on model diagnostics, the model was found to characterize well the ribavirin concentration–time data, and comparison of observed data and median and 90% prediction intervals of simulated data demonstrated close prediction over the time course of the study (Figure S2). Full details of the model performance are available as Supplementary data at *JAC* Online.

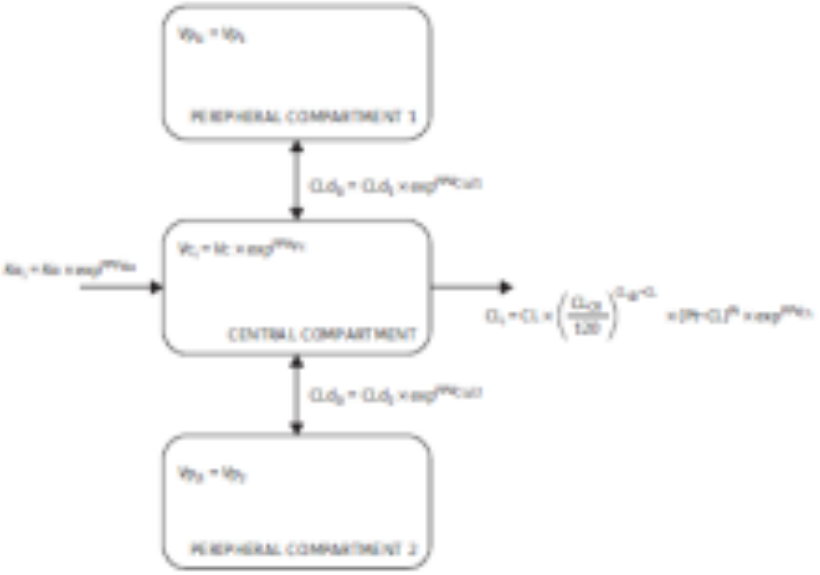

Figure 1. Schematic of the final rifavitrin population pharmacokinetic model. Cl_{CR}–Cl_r, effect of Cl_{CR} on Cl_r; Pt, patient type, where 0 = healthy control and 1 = lung transplant recipient; Pt–Cl, effect of patient type on Cl; PPV, population parameter variability.

Table 2. Final rifavitrin population pharmacokinetic model parameter estimates

Parameter	Final model estimate (RSE, %)	Shrinkage	Diff bar P
Fixed effects			
Cl (L/h)	17.5 (27.0)		
Vc (L)	92.2 (38.6)		
Vp1 (L)	152 (38.8)		
Vp2 (L)	1 140 (21.8)		
Cl$_{CR}$ (L/h)	40.7 (22.0)		
Cl$_{ex2}$ (L/h)	39.8 (37.5)		
Ka (h^{-1})	0.318 (19.8)		
F (%)	0.512 (32.1)		
Cl_{CR}–Cl	0.574 (84.6)		
Pt–Cl	0.586 (33.8)		
Random effects			
PPV Cl (CV, %)	36.9	39.5	0.759
PPV Vc (CV, %)	36.3	51.5	0.803
PPV Cl$_{CR}$ (CV, %)	34.6	49.9	0.787
PPV Cl$_{ex2}$ (CV, %)	69.4	26.3	0.842
PPV Ka (CV, %)	39.7	64.3	0.946
PPV F (CV, %)	66.8	1.3.8	0.958
Residual variability			
proportional (CV)	25.7	9.9	

Cl_{CR}–Cl_r, effect of Cl_{CR} on Cl_r; Pt–Cl, effect of patient type on Cl; PPV, population parameter variability; RSE, relative standard error; CV, coefficient of variation.

Examination of current and alternative dosing regimens

A representative patient population of 10000 lung transplant recipients with distributions of Cl_{CR} and associated body weights consistent with that seen within the patient cohort was constructed and concentration–time profiles were simulated using the developed population pharmacokinetic model to examine the ability of the current and alternative dosing regimens to achieve target concentrations over a 14 day treatment course. It should be noted that, for oral dosing regimens, doses administered were rounded to the nearest 300 mg (i.e. tablet size).

Both of the standard dosing regimens used within the study centres (11 mg/kg q8h orally or intravenously + 10 mg/kg q12h orally) performed well in achieving the predicted target concentrations within the first 24 h of starting; however, plasma rifavitrin concentrations continued to accumulate over the course of the treatment period such that >90% of patients were predicted to have concentrations well above the upper limit of the defined therapeutic range on day 14. Similar results were seen for the standard intravenous-only regimen (11 mg/kg q8h intravenously + 10 mg/kg q12h orally).[12] For the median patient, these terminal concentrations were predicted to be 2- to 3-fold higher than the target concentrations (Figure 2). Similar results were observed when stratified by renal function, indicating that the observed drug accumulation was not primarily due to altered Cl_{CR} in a specific patient subgroup (data not presented).

Alternative dosing regimens were examined to determine doses predicted to result in concentrations within the therapeutic range throughout the treatment course. An oral dosing regimen of

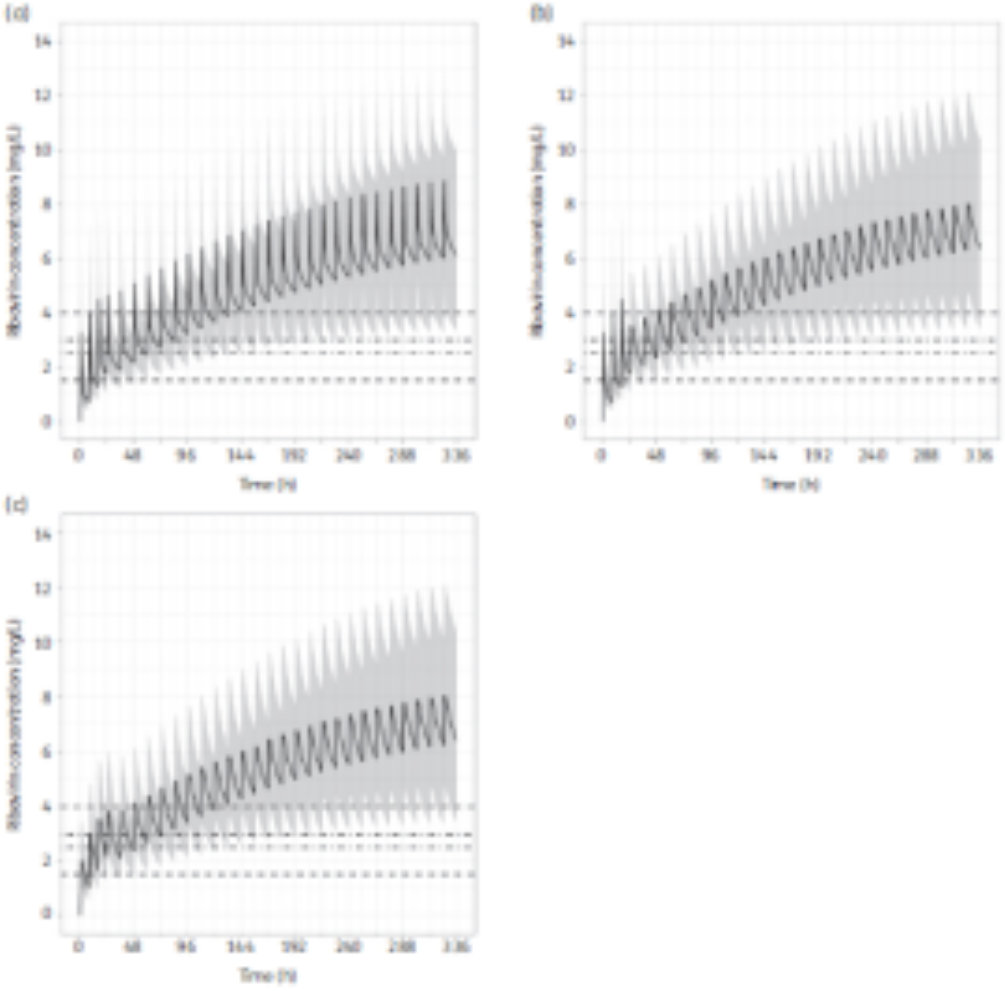

Figure 2. Plasma ribavirin concentration–time profile after administration of the standard ribavirin dosing regimens. Data are presented as median (solid line) and 90% prediction intervals (shaded). Target (2.5–3.0 mg/L, dot dashed line) and acceptable (1.5–4.0 mg/L, dashed line) therapeutic range denoted for reference. (a) 33 mg/kg q8h intravenously for the first 24 h, followed by a maintenance dose of 30 mg/kg q12h intravenously. (b) 11 mg/kg q8h intravenously for the first 24 h, followed by a maintenance dose of 10 mg/kg q12h orally. (c) 11 mg/kg q8h orally for the first 24 h, followed by a maintenance dose of 10 mg/kg q12h orally.

11 mg/kg q8h orally + 4 mg/kg q12h orally was found to result in plasma ribavirin concentrations for the median patient in the desired 2.5–3 mg/L range and >90% of patients achieving concentrations >1.5 mg/L at the end of the 14 day treatment period. However, despite the loading dose achieving therapeutic targets at the end of day 1, plasma concentrations declined on day 2 and then progressively increased by the end of the nominal treatment period (Figure 3c). Consistent results were seen across the spectrum of renal functions (data not presented). Similar results were seen for a dosing regimen of 11 mg/kg q8h orally + 8 mg/kg q24h orally (Figure 3b). On the other hand, a regimen of 8 mg/kg q8h orally for 48 h followed by 8 mg/kg q24h orally for the remainder of

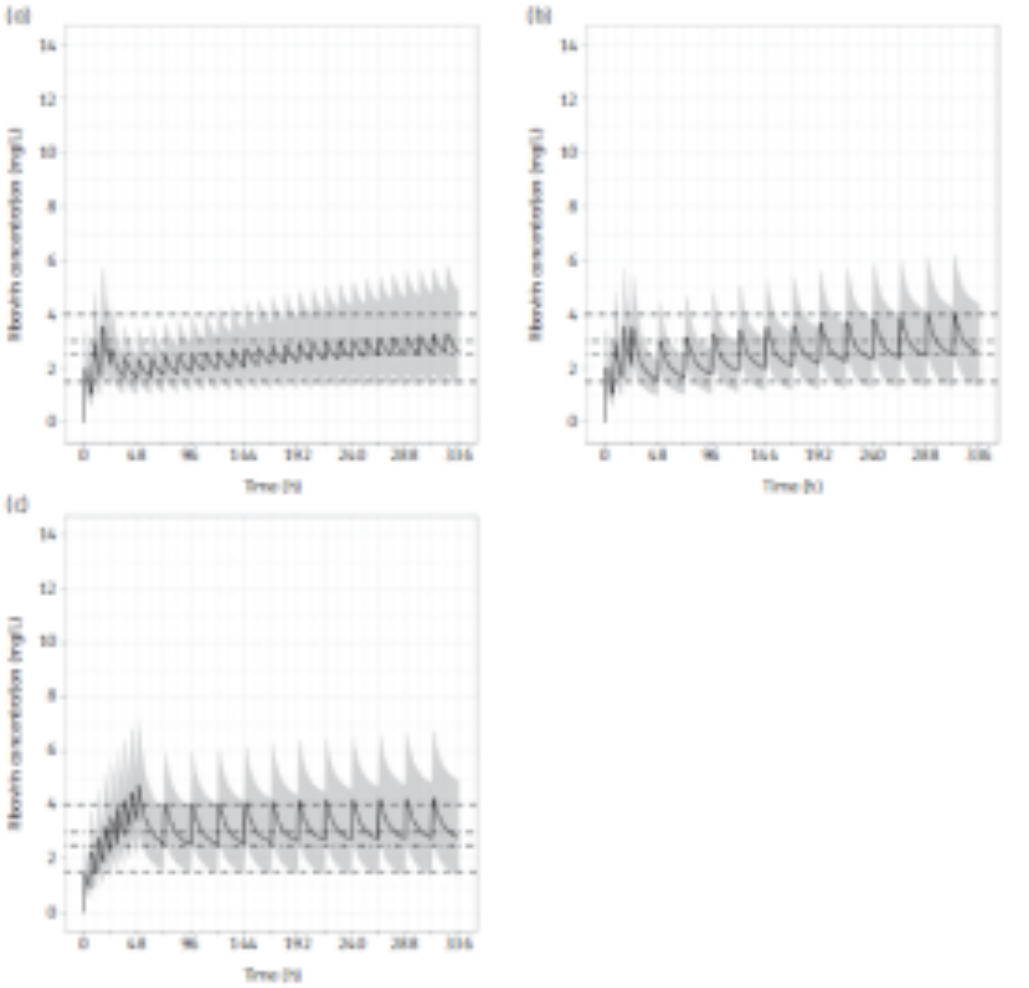

Figure 2. Plasma ribavirin concentration–time profile after administration of the alternative ribavirin dosing regimens. Data are presented as median (solid line) and 90% prediction intervals (shaded). Target (2.5–3.0 mg/L, dot dashed line) and acceptable (1.5–4.0 mg/L, dashed line) therapeutic range denoted for reference. (a) 11 mg/kg q8h orally for the first 24 h, followed by a maintenance dose of 4 mg/kg q12h orally. (b) 11 mg/kg q8h orally for the first 24 h, followed by a maintenance dose of 8 mg/kg q24h orally. (c) 8 mg/kg q8h orally for the first 48 h, followed by a maintenance dose of 8 mg/kg q24h orally.

the treatment period was predicted to result in >90% of patients exhibiting concentrations within the defined target range throughout the entire treatment course (Figure 3c). Slight drug accumulation was seen for patients with severe renal impairment (<30 mL/min, data not presented); however, concentrations were predicted to remained below those seen for the dosing regimens currently used in clinical practice.

Discussion

Despite the use of ribavirin for the treatment of paramyxovirus infections, little is known in regard to the ability of current dosing regimens to meet effective target concentrations. This is particularly relevant for the lung transplant population, for which sub-therapeutic treatment is likely to result in significant morbidity

and reduced median survival, and supra-therapeutic doses have been associated with haemolytic anaemia, renal impairment and other side effects.[14,16,17,24] Evidence-based dosing regimens are therefore critical for the optimal treatment of these patients. This study undertook a population pharmacokinetic approach to determine the pharmacokinetic properties of ribavirin and the factors contributing to variability in this patient group. This then allowed the examination of current and alternative dosing regimens and their ability to meet predefined therapeutic targets.

The population pharmacokinetic analysis confirmed that ribavirin is characterized by three-compartment pharmacokinetics, with first-order elimination, consistent with a compartmental analysis previously reported[25] and with the known pharmacokinetic properties of ribavirin that have been previously described.[14,15,18,19,26,27] The model found CL to be influenced by CL_{cr} (with a 10% reduction in CL_{cr} associated with a 6% reduction in systemic CL), which is not surprising given that renal CL accounts for approximately one-third of total CL.[11] Patient type also influenced CL, with lung transplant recipients exhibiting a 41% reduction in systemic CL compared with healthy controls.

Simulations of current intravenous and/or oral ribavirin dosing regimens indicate that, whilst administration of the standard loading doses results in plasma concentrations within the target therapeutic range early in the treatment course, concentrations continue to accumulate throughout the 14 day treatment period such that later concentrations are substantially higher than the defined upper limit of 4.0 mg/L (Figure 2). Previous studies indicated that ribavirin concentrations >3.5 mg/L are associated with severe side effects, even after only a few days of treatment, and thus it is feasible that these regimens may be associated with increased risk of side effects.[17,19,24] On the other hand, alternative dosing regimens that target effective concentrations at the end of the treatment period are predicted to result in sub-therapeutic concentrations during the first days of treatment (Figure 3), thereby potentially placing patients at risk of ineffective treatment. Utilizing the developed pharmacokinetic model and known characteristics of ribavirin, this study has been able to propose a dosing regimen, consisting of 8 mg/kg q8h orally for the first 48 h followed by a maintenance dose of 8 mg/kg q24h orally, that is predicted to result in early attainment of therapeutic concentrations and continued maintenance at these levels throughout the treatment course (Figure 3). Arguably, this indicates that therapeutic levels can be achieved with a 48% reduction in total dose administered; the combination of oral administration and dose reduction has potential for substantial cost savings. Notably, whilst this regimen includes higher loading doses, the predicted exposure remains below that observed later in the treatment period for the current regimens and therefore is considered to pose no additional risk of toxicity.

Whilst this study provides valuable information on the pharmacokinetics of ribavirin in the lung transplant population, the majority of patients within this study were recruited as outpatients and no severe inflammatory states were seen. Thus, the impact of this more complex clinical situation on ribavirin pharmacokinetics cannot be determined from this analysis. Furthermore, due to limited data and/or lack of information for some patients, the influence of factors such as underlying disease (including cystic fibrosis), time since transplantation and the immunosuppressive scheme could not be determined. Preliminary examination of the data indicated no discernible trends; however, full exploration with a larger dataset would be required for definitive conclusions to be made.

Importantly, it should be noted that current evidence for the proposed therapeutic target concentrations utilized within this study is limited and requires additional work. Longitudinal research examining the concentration–adverse effect relationship would be desirable in order to provide more evidence of the upper limit of ribavirin concentrations that can be tolerated for short-term therapy and how this relates to what is currently considered 'safe'. Nonetheless, the developed population pharmacokinetic model provides an effective tool for anticipating ribavirin exposure in a population with unique antimicrobial needs and providing an evidence basis for effective treatment protocols once target therapeutic concentrations are formally established. This study provides a valuable first step in determining optimal ribavirin treatment regimens for paramyxovirus infections in the lung transplant population.

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Transparency declarations

None to declare.

Supplementary data

Supplementary data, including Table S1, Appendix S1 and Figures S1 and S2, are available at *JAC* Online.

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CONFERENCE PRESENTATIONS

Milliken E, Marriott D, Schteinman A, de Zwart A, Sandaradura I, Carlos L, Burrows F, Glanville A, Reuter SE. A predictive pharmacokinetic model of ribavirin plasma concentration in lung transplant recipients [Poster Presentation]. International Congress of the Transplantation Society (TTS), August 2016: Hong Kong.

Abstract

Ribavirin is a guanosine analogue used for treatment of respiratory syncytial virus (RSV) pneumonitis in vulnerable populations, and is standard of care for RSV in global lung transplant centres to prevent acute pneumonitis and bronchiolitis obliterans syndrome. Despite this, the pharmacokinetic profile of ribavirin in the transplant population is unknown and is likely to be altered due to immunosuppressant regimens, renal and hepatic impairment and cystic fibrosis in affected individuals. At St Vincent's Hospital, the empirically-determined treatment protocol for ribavirin in lung transplant patients is three initial IV loading doses (11mg/kg tds) followed by maintenance dosing with oral ribavirin (10mg/kg bd); however, whilst the treatment protocol has demonstrated efficacy, the ribavirin exposure for the two treatment formulations has not been established. To examine the pharmacokinetics of oral and IV ribavirin in the lung transplant population, a prospective, single-centre cohort study was conducted. Twelve lung transplant patients with PCR-confirmed RSV or HMPV infection were recruited for study participation (7 male/5 female; Age: 45.3 ± 13.9 years; BMI: 23.8 ± 4.82 kg/m^2; CrCL: 69.2 ± 26.7mL/min). Patients were administered ribavirin according to the standard hospital protocol, and blood samples were collected throughout the IV and oral treatment periods. Plasma ribavirin concentrations were quantified using a validated HPLC-UV analytical method. Patient concentration-time data, combined with previously published ribavirin pharmacokinetic data, were used to develop a population pharmacokinetic model, using NONMEM® VII software, incorporating inter-individual and residual unexplained variability. Patient factors contributing to parameter variability (such as renal function, patient status, body weight) were modelled using standard forward-inclusion/backward-deletion methods. Model selection was based on the objective function value and standard diagnostic plots. The developed model

was then used to conduct Monte Carlo simulations examine alternate dosing regimens, in particular if comparable drug exposure can be achieved with oral only dosing regimens as opposed to mixed regimens, thereby reducing patient bed days and increasing convenience. Additional research is needed to establish effective plasma ribavirin concentrations for viral eradication; however, it is anticipated that the developed pharmacokinetic model will allow predictions of optimal dosing regimens to meet therapeutic pharmacokinetic/pharmacodynamic endpoints as more is learned about effective treatment of respiratory viruses in lung transplant patients.

Milliken E, de Zwart AES, Alffenaar JC, Marriott DJE, Riezebos-Brilman A, Schteinman A, Evans AM, Glanville AR, Verschuuren EAM, Reuter SE. Population pharmacokinetics of ribavirin in lung transplant recipients and examination of current and alternative dosing regimens [Oral Presentation]. International Congress of Therapeutic Drug Monitoring & Clinical Toxicology (IATDMCT), September 2018: Brisbane, Australia.

Abstract

The guanosine analogue ribavirin (RBV) is an established treatment for respiratory viruses in lung transplant recipients. [1-5]. Goals of treatment are prevention of progression of lower respiratory tract viral infection to deadly pneumonitis as well as prevention of the bronchiolitis obliterans syndrome, linked with progressive deterioration of graft function [5-7]. Despite RBV's common usage, pharmacokinetic data is limited and the difference in relative exposure between oral and intravenous regiments, both of which are used at transplant centres internationally, is unknown. To address this the authors developed a physiological pharmacokinetic model of RBV in the transplant population using data collected from a cohort of lung transplant recipients being treated with RBV for respiratory syncytial virus (RSV), human metapneumovirus (HMPV) or parainfluenza virus infection. Data was collected at St Vincent's Hospital Sydney and Groningen University Medical Centre, Netherlands. The model was validated using published data from healthy volunteers [8]. Our model established that there is no difference in the pharmacokinetics of RBV in lung transplant recipients in comparison to the general population. It also support PO only regimes for a desired plasma concentration of 1.5-3.0 μg/mL as well as providing a valuable tool for predicting effective dosing in this vulnerable population.

Milliken E, de Zwart AES, Alffenaar JC, Marriott DJE, Riezebos-Brilman A, Schteinman A, Evans AM, Glanville AR, Verschuuren EAM, Reuter SE. Population pharmacokinetics of ribavirin in lung transplant recipients and examination of current and alternative dosing regimens [Poster Presentation]. Australasian Pharmaceutical Science Association (APSA) Annual Conference, December 2018: Adelaide, Australia

Abstract

Introduction. Ribavirin is used in the treatment of respiratory paramyxovirus infection in lung transplant recipients; however, its pharmacokinetic profile in the transplant population is unknown despite the potential for alterations due to underlying pathology. Furthermore, the ability of current regimens to meet exposure targets has not been established. Aim. This study examined the pharmacokinetics of ribavirin in a lung transplant population from which current and alternate dosing regimens were assessed. Methods. Population pharmacokinetic modelling was conducted in NONMEM using concentration-time data from 24 lung transplant recipients and 6 healthy volunteers. Monte Carlo simulation was used to assess the ability of dosing regimens to achieve pre-specified target concentrations. Results and Conclusions. A three-compartment model with first order elimination most adequately described ribavirin concentration-time data, with creatinine clearance and patient type (i.e. lung transplant) identified as significant covariates in the model. Simulations indicate that current regimens achieve efficacious concentrations within 24 hours of treatment initiation that increase to supra-therapeutic levels over the treatment period. A regimen of 8 mg/kg q6h oral for 48 hours followed by 8 mg/kg q24h oral for the remainder of the treatment period was predicted to result in >90% of patients exhibiting concentrations within the defined target range throughout the entire treatment course. Additional work to formally establish of target therapeutic concentrations is required; however, this study provides a valuable first step in determining optimal ribavirin treatment regimens for paramyxovirus infections in the lung transplant population.

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