New Analytical Techniques for Determining Pharmacokinetics of Drugs in Neonates

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Declarations

Statement of Originality

I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision. The thesis contains no material which has been accepted, or is being examined, for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968 and any approved embargo.

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In the end, this is *FOR LUKE*, without whom nothing I achieve would be possible.

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O'Hara K. *Pharmacokinetic changes with growth and development between birth and adulthood.* **Journal of Pharmacy Practice and Research** 2017;47:313-318.

O'Hara K. *Paediatric pharmacokinetics and drug doses*. **Australian Prescriber** 2016;39(6):208-210.

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Abstract

Determining the appropriate dose of medication to use in a paediatric or neonatal patient is a clinical challenge. Many doses of medication currently used are extrapolated from adult dosing regimens due to a lack of pharmacokinetic studies in children and neonates. The lack of paediatric clinical trials and dosing information has been highlighted by many different international bodies, including the Food and Drug Administration (FDA) and European Medicines Agency (EMA) who acknowledged that this is an area of clinical need and there is now a requirement for more paediatric data in the licensing of new drugs. There is an urgent need for pharmacokinetic studies to be performed in paediatric and neonatal patients. Pharmacokinetic studies require the availability of a suitable assay to measure the drug concentration in blood or plasma. Without these analytical techniques, it is impossible to perform the required pharmacokinetic studies to develop dosing information in these patient groups. Neonates, in particular, have very small total blood volume and any samples taken must reflect this. The primary aim of this thesis is to describe the complex development of analytical techniques capable of measuring drug concentrations in small volume blood samples. Addressing this lack of suitable assays is a critical first step in pharmacokinetic research in this patient group.

As part of the research performed for this thesis, a clinical study in neonates was conducted. From the experience of designing and conducting this clinical study in neonates, combined with a review of the literature, several barriers to this type of research were identified. These barriers included gaining ethics approval, parental consent issues, sufficient number of patients and multicentre trials, minimising blood sampling requirements and availability of suitable analytical techniques. Reflecting on these identified barriers, potential solutions to overcome these barriers have been proposed to assist researchers in the future.

Similar to the adult population, the pharmacological therapies in neonatal patients span a huge range of medical conditions and illnesses. As a result of the review conducted for this thesis, priority areas requiring pharmacokinetic studies and further work were identified. This included analgesia, antibiotics and sedatives. In this thesis, small volume assays for drugs with clinical significance for neonatal patients in each of these areas were developed and validated as a first step towards determining pharmacokinetic data and evidence based dosing information.

Remifentanil has been identified as a potentially useful analgesic in neonates. In this research, an assay capable of measuring concentrations as low as 0.25ng/mL in 100µL of plasma was developed using HPLC-MS/MS. Applicability to use in pharmacokinetic studies was demonstrated by analysing small volume samples to determine pharmacokinetics in a rabbit model. Remifentanil undergoes metabolism via hydrolysis by esterases. Few data are available about extent of esterase activity in neonates. Work in this project explored esterase activity in neonatal red blood cells and plasma. This research provided data not previously reported in the literature on the extent of metabolism of remifentanil in neonatal blood. It was able to demonstrate that developmental changes are likely to occur and extrapolating dosing information from adults may present dangers to neonatal patients.

Neonates often require antimicrobial therapy and benzylpenicillin is a commonly used agent in this group. Detailed pharmacokinetic data would assist in optimising dosing, particularly with concerns about antibiotic resistance. An analytical technique capable of measuring concentrations as low as 10ng/mL in 50µL of plasma was developed and validated. This assay uses HPLC-MS/MS and would enable quick turnaround in analysis, making it potentially suitable for both clinical pharmacokinetic studies and therapeutic drug monitoring. As sample stability is a concern in previous benzylpenicillin studies this is also addressed. Contrary to some previously reported data in the literature,

benzylpenicillin was observed to be stable for up to 24 hours at room temperature, providing information to determine appropriate sample collection for clinical studies.

Providing adequate sedation is a challenge in neonates. The benzodiazepine, midazolam, has been used but little is known about its pharmacokinetics and the optimal dose. It is metabolised by CYP3A4, which is known to undergo developmental changes. In order for future clinical pharmacokinetic studies, as assay that would be suitable for use in a neonatal pharmacokinetic study using a limited sampling strategy was developed using HPLC-UV detection. The assay requires a 300μL plasma sample and is capable of measuring concentrations a slow as 10ng/mL. The applicability of this assay for use in neonates was demonstrated by performing pharmacokinetic analysis in a rabbit model.

This thesis outlines the experimental difficulties that must be overcome to develop practical and accurate analytical techniques, and develops methods to overcome some of the analytical barriers. Specifically, in the following chapters different strategies to overcome these difficulties are described including sample stability, accurate small volume detection and use of HPLC-MS/MS technology. Practical application of the analytical techniques is shown in analysis of midazolam in rabbit samples, benzylpenicillin in simulated patient samples to determine stability and remifentanil in a rabbit pharmacokinetic study and an in-vitro assay to determine differences in the rate of metabolism between adults and neonates.

Outline

Chapter 1 describes the unique pharmacokinetics of neonates along with the research tools and methods needed to conduct pharmacokinetic studies in neonates.

Chapter 2 outlines the difficulties in designing and developing neonatal pharmacokinetic studies and describes the solutions used throughout this work that will be of use to other researchers in the field.

The development of a low volume highly sensitive assay for remifentanil in blood samples is covered in Chapter 3. This assay was designed for use with neonatal patients and clinical applicability is demonstrated in rabbits.

Developing population pharmacokinetic models for remifentanil dosing in neonates is complicated by a lack of knowledge of the activity of remifentanil metabolising non-specific esterases in this age group and how they may differ from adults. Chapter 4 is a preliminary study of the differences in esterase metabolism in blood between the two groups to gather information for use in developing safer dosing information.

Benzylpenicillin is a commonly used antibiotic for treating infections in neonates. Chapter 5 describes the development of a low volume highly sensitive assay for determining benzylpenicillin concentrations in clinical samples. This chapter also includes a sample stability study to assist in collecting data from clinical samples. The work described in this chapter also highlights the need for appropriate analytical equipment for analysing neonatal samples by describing the failures during method development.

Development of a midazolam assay that is suitable for use in neonates is described in Chapter 6. This assay was applied clinically by completing a pharmacokinetic study in rabbits, which are able to provide similar blood sampling volumes to neonatal patients.

Chapter 7 discusses the relevance and future directions of this body of work.

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List of abbreviations

CBP	Cardiopulmonary Bypass
CNS	Central Nervous System
CYP	cytochrome enzyme
CYP450	cytochrome P450
EMA	European Medicines Agency
FDA	Food and Drug Administration
GABA	gamma-Aminobutyric acid
HPLC	High Performance Liquid Chromatography
IS	Internal Standard
IV	Intravenous
LLOQ	Lower limit of Quantitation
mL	millilitre
MS	Mass Spectrometry
ng	nanogram
NICU	Neonatal Intensive Care Unit
PICC	Peripherally Inserted Central Catheter
QC	Quality Control
SPE	Solid phase extraction
TDM	Therapeutic Drug Monitoring
TGA	Therapeutic Goods Administration
UGT	UDP-glucuronosyltransferase
UV	Ultraviolet
µg	microgram
µl	microliter