PAEDIATRIC FORMULATIONS

Pharmaceutical Development and Clinical Evaluation

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PAEDIATRIC FORMULATIONS
Pharmaceutical Development and Clinical Evaluation

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Farmaceutische Ontwikkeling en Klinische Evaluatie

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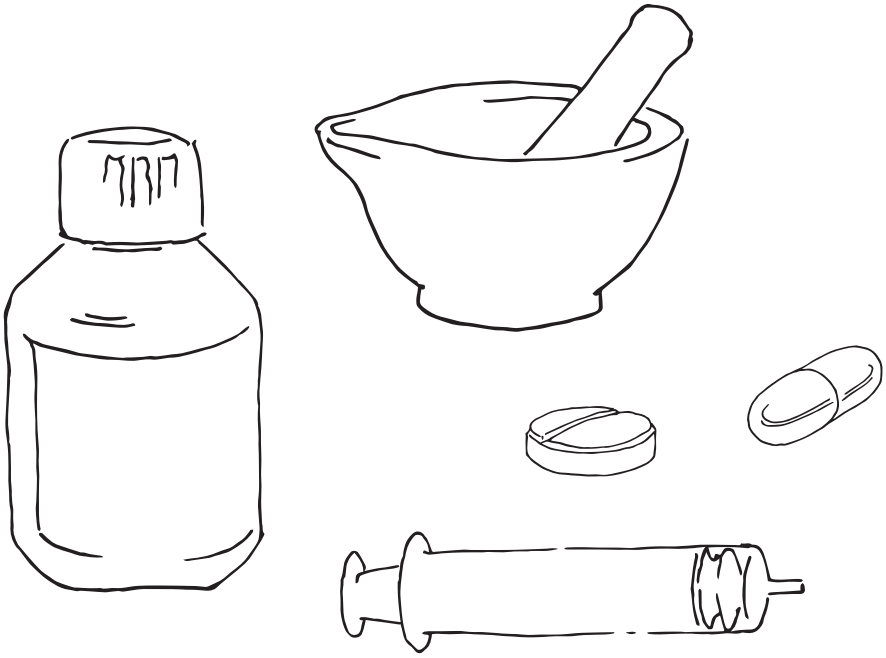
PART I

Unfulfilled needs and poor practices relating to pharmaceutical products applied in paediatrics in daily clinical practice



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Introduction

The development of medicines for children has long been a neglected area. Until late into the 20th century, the general view was that, for ethical reasons, children should not be subjected to clinical research. Nowadays, the consensus is that children are entitled to medicines that have been appropriately evaluated for their use, but other barriers still remain. As the paediatric population from premature neonate to adolescent is very heterogeneous, it cannot be approached as a uniform group. This brings not only practical issues in study design, but the smaller populations also mean a lower returns on investment for companies. As a result, a paucity exists in medicines designed and studied for use in children. On a European level, at the end of 2006, of the 317 centrally authorised medicines, 43% had a potential paediatric use, but were not authorised in this manner (1).

European legislation and incentives for the development of paediatric medicines

Within the European Union, this paucity in paediatric medicines was acted upon by specific legislation in the form of the Paediatric Regulation (EC No 1902/2006), following the example of the US Best Pharmaceuticals for Children Act. When this regulation came into effect in 2007, one of the first measures that were taken was the establishment of the Paediatric Committee, with its main role of scientific assessments and agreement of paediatric investigation plans (PIP). Since then, all applications for marketing authorisation for new medicines have to include the results of studies as described in an agreed PIP, unless the medicine is exempt because of a deferral or waiver. This has resulted in 949 agreed PIPs by the end of 2016, of which 131 had been completed (2). Between the adoption of the Paediatric Regulation in 2007 and the end of 2016, 101 of 399 (26%) centrally authorised new medicines received a paediatric indication. The Paediatric Regulation is therefore seen as successful, but the above applies mainly to innovative medicines, and does not include the development of off-patent medicines.

To stimulate the development of off-patent medicines for paediatric patients, several measures were taken. Firstly, the Paediatric Use Marketing Authorisation (PUMA) was established by Article 30 of the Paediatric Regulation. It is an incentive for off-patent medicinal product development for paediatric use, which offers 10 years of data and marketing protection. Secondly, specific European funding for research into off-patent medicinal products was made available, for instance through the EU Framework Programmes for Research and Technological Development. Thirdly, an inventory of paediatric needs was made, which is published on the EMA website (3), and is meant to help developers identify opportunities. It consists of lists of medicines by therapeutic class, which identify needs with respect to clinical data and age appropriate formulations. From these lists, it is evident that there is a great lack of age-appropriate formulations for off-patent medicines. Unfortunately, up to 2018, only four PUMAs have been granted (4), and it seems that the data and marketing protection is not an effective incentive.

The role of pharmacists in supplying paediatric patients with age-appropriate formulations

Even though the development of new medicines has improved greatly since the introduction of the Paediatric Regulation, there are many therapeutic areas in which there is still a need for paediatric formulations of older medicines. When age-appropriate licensed formulations are not available, pharmacists have several options in providing paediatric patients with suitable preparations. The most preferred option would be to seek a licensed therapeutic alternative. Examples of drug classes where substitution is common

are proton pump inhibitors and NSAIDs. Importation of products that are authorised in another EU country is a second option, but this can be time consuming and costly, and is often subject to strict regulations, which are country-specific. In the Netherlands, reimbursement is also difficult for non-licensed imported products. A third option is the compounding of medicines within the pharmacy, defined as the preparation of an unlicensed medicine to meet the specific needs of a patient. This can either be using raw materials, or the authorised dosage form. These three options are much preferred above the alternative; the manipulation of licensed dosage forms, such as splitting or crushing of tablets, or mixing with fluids or food, by parents and caregivers. With this option, the risk of quality issues is probable, and bioavailability may be substantially altered. When crushing Kaletra (lopinavir/ritonavir) tablets for example, lopinavir and ritonavir exposure in children reduced by 45% and 47%, respectively (5).

Officially, two types of pharmacy preparations are recognised in Directive 2001/83/EC, known as **magistral** formulae (any medicinal product prepared in a pharmacy in accordance with a medical prescription for an individual patient) and **officinal** formulae (any medicinal product which is prepared in a pharmacy in accordance with the prescriptions of a pharmacopoeia and is intended to be supplied directly to the patients served by the pharmacy in question). In The Netherlands, as in several other European Member States, an alternative practice is common, where centralised, GMP-certified pharmacies manufacture unlicensed medicines and supply them to local pharmacies. Although in conflict with Directive 2001/83/EC, it is officially allowed by the Health and Youth Care Inspectorate because of the obvious improvement in pharmaceutical quality it provides, but it is tightly regulated.

Practices concerning compounding/manufacturing of unlicensed paediatric formulations and the facilities and equipment available to pharmacists are highly variable across the European Union. In an effort to standardise quality and availability throughout the EU, initiatives are currently undertaken towards the compilation of a pan-European Paediatric Formulary, consisting of monographs for extemporaneous formulations, based on national or regional information. Led by the European Committee on Pharmaceuticals and Pharmaceutical Care (CD-P-PH) and the European Pharmacopoeia Commission, a working party of European experts is currently working on the selection and elaboration of the formulations to be included (6). It is expected that the Formulary of Dutch Pharmacists (FNA) will contribute largely to this Paediatric Formulary.

Paediatric product development

Most of the unlicensed products dispensed to paediatric inpatients are manufactured at GMP-pharmacies, and are thus based on pharmaceutical quality data and extensive product dossiers. This also applies to the two drug products presented in this thesis, which were designed at the pharmacy of the Erasmus MC and studied in association with the Laboratory of Dutch Pharmacists (LNA). The LNA is a department of the Royal Dutch Pharmacists Association and supports pharmacist in the compounding of essential medicines of good quality, when licensed products are not available.

The starting point of product development for new paediatric products is always the clinical need. Generally, therapeutic rationale has been established, but the available dosage forms fall short. The EMA has offered some guidance for the selection of dosage forms in relation to the acceptability by paediatric patients, summarised in a reflection

paper (7). One of the main considerations is the ability to deliver the correct dose to the patient. Within the heterogeneous paediatric population, this means that dosing flexibility is required for a specific drug, and it reduces the options to low-dose solid dosage forms, liquids or parenteral formulations. In the inpatient setting, as a large proportion of the patients is below the age of two or is dependent on a feeding tube, liquid formulations are usually the first choice if non-parenteral administration is aimed for. In addition to the standard drug and formulation properties such as dosage strength, solubility, taste and stability, certain aspects of the formulation need specific attention when designing a product for paediatric patients, in particular the choice of excipients. The EMA guideline on pharmaceutical development of medicines for paediatric use (EMA/CHMP/QWP/805880/2012) offers useful guidance for the selection of excipients, and a hierarchized list of information sources to consult in order to assess the safety profile of each one. Another important property to consider is the palatability of the excipients and the drug product as a whole. Palatability, a combination of taste, after-taste, mouth-feel, fragrance and appearance, is one of the main elements determining the acceptability of paediatric medicinal products (8).

In vitro evaluation of paediatric products

Currently, most *officinal* formulae that are compounded or manufactured in the Netherlands and applied in paediatrics, have not been clinically evaluated. This has led to unexpected deviations in exposure to the drug in multiple occasions, an example being the reduced oral bioavailability of tacrolimus suspension, compared to tacrolimus capsules (9). Ideally, in the future, *in vivo* performance of oral dosage forms in children can be predicted with use of *in vitro* biopharmaceutical techniques. Unfortunately, the drug absorption processes in children have not yet been sufficiently elucidated to develop and validate accurate biopharmaceutical methods.

In vivo studies

When formulation development has been completed, sometimes it is necessary evaluate the product *in vivo*. A general principle is that paediatric patients should be given medicines that have been appropriately evaluated for their use. Unnecessary clinical trials in (paediatric) patients should however be avoided. From a regulatory perspective, a new formulation that has not been tested in efficacy trials, requires a bioequivalence study, which should typically be performed in adults (10).

Bioequivalence studies are performed to make sure that two formulations have the same rate and extent of absorption (within predefined limits), to ensure comparable *in vivo* drug exposure. The parameters area under the curve (AUC), maximum plasma concentration (C_{max}) and sometimes time to maximum plasma concentration (t_{max}), are calculated from dense sampling schemes and compared between formulations. Bioequivalence studies may however be exempted, if *in vitro* data can be expected to adequately predict the *in vivo* performance. These so-called biowavers are based on the Biopharmaceutic Classification System (BCS, Figure 1). The BCS is a system to differentiate drugs on the basis of their solubility and permeability (11). A drug is considered highly soluble when the highest dose strength is soluble in 250 ml or less of aqueous media over the pH range of 1 to 7.5. A drug is considered highly permeable when the extent of absorption in humans is determined to be 90% or more of the administered dose based on a mass-balance determination or in comparison to an intravenous dose. BCS class 1 (highly soluble and

highly permeable) and sometimes class 3 drugs (highly soluble and low permeable) are eligible for biowaivers. Additional conditions for a biowaiver are rapid dissolution and similar excipients, if they might affect the bioavailability.

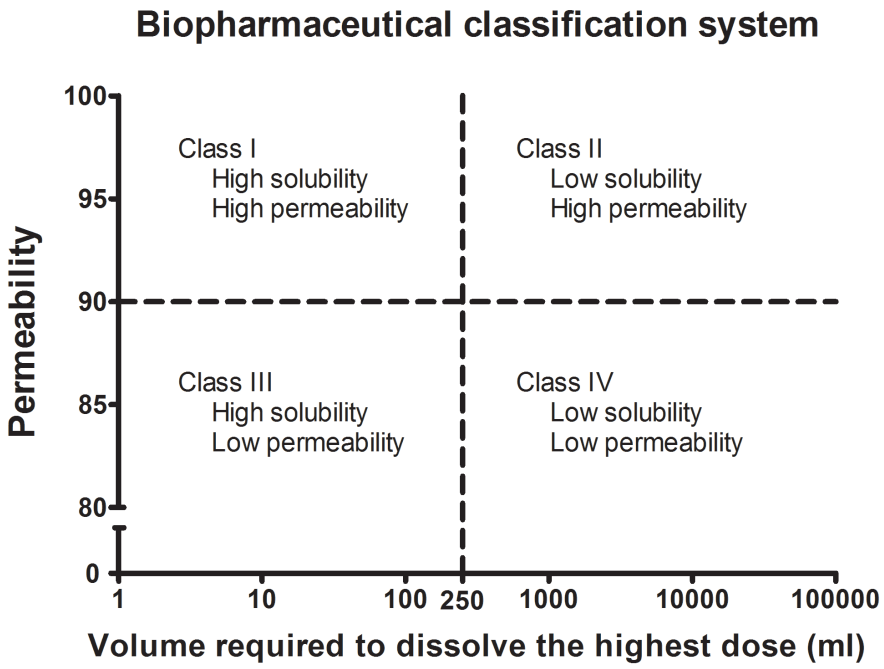

Figure 1.1 Biopharmaceutical classification system. The x-axis shows the volume (ml) required to dissolve the highest dose strength of the drug at the lowest solubility over the pH range 1–7.5. Permeability is defined by various *in vivo* or *in vitro* assays. A drug is considered highly permeable when the extent of oral absorption in humans is determined to be 90% or more of the administered dose based on a mass-balance determination or in comparison to an intravenous dose.

When it comes to paediatric formulations, there are some limitations to this approach. Both the parameters solubility and permeability may not be extrapolated to paediatric population. Consequently, BCS-based biowaivers, as well as adult bioequivalence studies, need to be regarded with caution in the paediatric setting.

Aims and outline of this thesis

This thesis we describe the work that was carried out towards a framework for the development of paediatric oral liquids and their evaluation in the target population.

In part one of this thesis we aimed to identify the unfulfilled needs and poor practices relating to pharmaceutical products applied in paediatrics in daily clinical practice. It has two main focus points: firstly, the availability and suitability of drug products for paediatric patients, and secondly, the practical issues regarding administration of drug products to paediatric patients. In chapter 2 we describe studies into the drug products that were dispensed from the pharmacy and assessed their suitability for the specific patient according to EMA guidelines. Furthermore, we identified liquid drug products that are unsuitable due to the presence of potentially harmful excipients, based on the extent of exposure. In chapter 3 we surveyed the extent of manipulation of drug products required to adequately administer the drug to the patient. Both parents and nurses were involved in the study, using questionnaires (parents) and observation (nurses) as main methods.

The second part of this thesis contains the formulation development that was conducted in collaboration with the Laboratory of Dutch Pharmacists. For children, oral liquid formulations with acceptable palatability, good pharmaceutical quality and possibility of flexible dosing are still urgently needed. As a proof of concept, two drugs were selected, both frequently used in children; amlodipine representing a typical BCS class I drug, and lorazepam as an example of a drug with poor aqueous solubility. In chapter 4 we describes the pharmaceutical development of an amlodipine 0.5 mg/ml oral liquid, and chapter 5 proposes a liquid formulation for poorly soluble compounds with lorazepam as a proof of concept. Chapter 6, which was a collaboration with the University of Bath, explores *in vitro* biopharmaceutical methods that could be used to predict formulation performance in paediatric patients.

In the third part of this thesis we present the clinical studies that were conducted following the pharmaceutical development of the two experimental formulations. Chapter 7 contains the results of a bioequivalence trial in adults of commercial amlodipine tablets and the oral liquid described in chapter 4. This liquid was subsequently studied in the target population using a population pharmacokinetic design. The retrospective study in chapter 9 evaluates the effects of an IV midazolam to oral lorazepam conversion on withdrawal and sedation levels on the paediatric intensive care unit. The subsequent clinical trial in which the bioavailability and pharmacokinetics of our lorazepam oral liquid is studied is described in chapter 10.

Finally, the results, conclusions and recommendation from the studies described in this thesis are discussed in a summarizing discussion.

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Availability of age-appropriate paediatric formulations; the need in daily clinical practice remains

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Manuscript in preparation

ABSTRACT

Objectives

To quantify the availability of authorised, age-appropriate paediatric medicines in clinical practice and to identify gaps.

Methods

The availability of age-appropriate formulations was assessed by conducting a survey on the use of pharmacy compounded medicines among the paediatric hospitals in the Netherlands, and by analysing dispensing data of oral medication from the inpatient pharmacy of the largest paediatric hospital in the Netherlands. The age-appropriateness of the dispensed formulations was assessed on two aspects: dose-capability and acceptability. Liquid drug products that are unsuitable due to the presence of potentially harmful excipients, were identified based on the dosage in clinical practice.

Results

For 129 out of 139 drug substances included in the survey (93%), at least one of the eight respondents stated to use a pharmacy compounded product to meet the needs of their paediatric patients. The age-appropriateness of medicines dispensed form the inpatient pharmacy increased with age, and was higher for non-ICU patients than for ICU patients. We identified 15 drug products causing excipient exposure above the EMA recommended values.

Conclusions

This study confirms there is still a large need for age-appropriate formulations in daily clinical practice. Pharmacy compounding for paediatric patients remains essential for many indications.

INTRODUCTION

Drug development for children has long been a neglected area compared to adult drug development. Low prevalence of disease and the resulting low return on investment, together with ethical and practical barriers, have not been providing enough incentives for pharmaceutical corporations to invest time and resources into bringing appropriately tested paediatric medicines to the market. It was recognised that specific legislation was needed to address this issue. Following the example of the US Best Pharmaceuticals for Children Act, the EU Paediatric Regulation (EC) No 1901/2006 was adopted in December 2006 (1).

Since the introduction of the Paediatric Regulation, many initiatives have been taken to improve the availability of paediatric drug formulations. New dosage forms such as dispersible films and multi-particulates (sprinkles, mini-tablets e.g.) have been developed (2), and during the years 2007-2016, over 260 new medicines have been authorised in the EU for use in children, which is regarded as the success of the Paediatric Regulation (3). Unfortunately, we also see that the paediatric use marketing authorisation (PUMA) failed to deliver age-appropriate paediatric formulations for off-patent drugs, with only four PUMAs granted so far (4) .

Looking at the European Medicines Agency (EMA) priority list of off-patent medicinal products (5), and the inventory of needs for paediatric medicines (6), a discrepancy emerges between the availability of marketed paediatric medicines and the medicines needed in daily practice. Within the Netherlands, the limited commercial availability of authorised medicines for children has previously been recognized by van Riet-Nales et al (7). These authors compared dosing information for use in children from a national Medicines Compendium (Informatorium Medicamentorum) with the official indications in the Summary of Product Characteristics (SmPC), and found a 48% overall availability of authorised medicines for children. Furthermore, the age-appropriateness of the formulation, as well as the presence of potentially harmful excipients were assessed, confirming a lag in pharmacotherapeutic treatment options compared to adults. However, this study did not involve the need in clinical practice in its design.

The absence of age-appropriate, authorised and commercially available dosage forms is forcing pharmacists to compound drugs, or caregivers to manipulate adult formulations before administration. Individual compounding and manipulation of medicines can be costly and time-consuming, but most importantly carry risks for the patient. Examples of safety issues linked to compounding include decreased bioavailability of a tacrolimus suspension (8), and a 10-fold dosing error of spironolactone due to the availability of different strengths (9). Manipulations such as crushing of tablets can lead to loss of controlled release properties, or loss of drug substance (10). Another important disadvantage of the use of unstandardized compounded medicines is the absence of clinical decision support with electronic prescribing.

Regardless of the authorisation status, a lot of medicines dispensed to paediatric patients are not age-appropriate, either because of unacceptability of the dosage form to the patient or because of incapability to provide the correct dose (7). The acceptability of different dosage forms to paediatric patients has been summarized in the 'Reflection paper on formulations of choice for the paediatric population' by the EMA (11). It provides a matrix proposing applicability and acceptability of different dosage forms in specific age

groups. It was presented as a rough guide, and not an evidence-based recommendation for the development of dosage forms. Since then, acceptability studies of different dosage forms have become available, but the methodologies have not been standardized, and for some age groups and dosage forms, no consensus has been obtained (12).

One aspect determining the age-appropriateness of medicines is the presence of potentially harmful excipients. Excipients are generally considered to be pharmacologically inactive, but they pose a risk for patients with immature metabolic pathways and organ systems. For several of them, the EMA has published recommendations advising maximum daily doses which are considered to be safe (13). These potentially harmful excipients are frequently used in liquid formulations, but their harmfulness is relative to exposure and patient characteristics. Excipient exposure in preterm infants and neonates has previously been assessed for several substances (14-16). These studies showed that a lot of drug products used in paediatrics are possibly unsuitable due to their excipients, but to date, this has only been evaluated for the youngest patients.

In summary, there is still a limited availability of commercial and age-appropriate paediatric medicines, but the magnitude of the problem in clinical practice has not been determined. The aim of this research was to quantify the availability of commercial, age-appropriate paediatric medicines and to identify gaps. In order to achieve this aim, we made use of different strategies and datasets and in contrast to earlier work, this study specifically focuses on daily clinical practice.

The availability of commercial drug products, restricted to oral medication, was assessed using two datasets; 1) a survey on the use of pharmacy compounded (non-commercial) medicines among the paediatric hospitals in the Netherlands and 2) dispensing data from the inpatient pharmacy of the largest paediatric hospital in the Netherlands. Subsequently, the age-appropriateness of the dispensed oral formulations was assessed according to EMA acceptability guidance and additional criteria previously applied by Van Riet-Nales et al (7). Finally, we identified liquid drug products that are unsuitable due to the presence of potentially harmful excipients, based on the extent of exposure in clinical practice. The results of these strategies were combined to find the gaps in the availability of age-appropriate paediatric formulations. These gaps can form the agenda to develop additional age-appropriate flexible dosage forms.

METHODS

1. Availability of paediatric medicines in the Netherlands

In 2016, a survey was conducted among the Dutch academic and teaching paediatric hospitals, to identify the use of pharmacy compounded medicines for paediatric patients in the Netherlands. For the survey, we established a list of drugs of interest based on the existing monographs of the Dutch Paediatric Formulary (17). Based on route of administration (oral), unavailability of a commercial oral liquid dosage form, and the absence of an equivalent therapeutic alternative (e.g. pantoprazole and omeprazole), we included 139 drug substances (Appendix 1) in the survey. Respondents were asked to confirm if 1) the drug was applied for their patients, 2) a commercially available product was able to meet the needs of their patients, and 3) they made use of a pharmacy compounded product. Furthermore we asked them to supplement the list with any products they thought were missing. The results were subsequently compared with the

EMA inventory of paediatric needs.

To supplement the qualitative data collected in the survey, we used the prescription and dispensing data of the Erasmus MC-Sophia Children's Hospital to quantify for which age groups dispensing of pharmacy compounded, non-commercial products was most prevalent. In this dataset, non-formulary medicines were also included. Age categories were defined according to the guideline on clinical investigation of medicinal products in the paediatric population (18), and all patients admitted to the NICU were categorised as preterm neonates. Prospective data collection by a MSc pharmacy student took place at the inpatient pharmacy on weekdays over a period of 10 weeks during the autumn of 2016. All electronically prescribed medication orders, for patients admitted to the Paediatric Intensive Care Unit (PICU), Neonatal Intensive Care Unit (NICU) or the remaining non-ICU units (surgical, oncology, and general wards) were evaluated at start of treatment. The electronic prescription data were corrected when the dispensed dosage form deviated from the prescription.

2. Age-appropriateness of paediatric formulations dispensed from the inpatient pharmacy

In addition to the availability of commercial drug products, we evaluated the dose-capability (the capability to deliver the correct dose) and age-appropriateness of all oral medication dispensed from the pharmacy according the criteria previously applied by Van Riet-Nales *et al* (7), using the dispensing dataset described above. In this dataset we also included injections fluids dispensed for oral administration. To assess dose-capability, manipulations to the dispensed product required to obtain the correct dose, such as tablet splitting, were verified with the SmPC. Age-appropriateness of the formulation was determined using the acceptability matrix of the EMA reflection paper (11), applying the criteria that a value of 4 or 5, combined with the additional criteria displayed in Table 2.1, represent sufficient suitability. Different from Van Riet-Nales *et al,* we considered capsules that may be opened, and tablets that may be pulverized according to the SmPC, to be suitable for children from the age of 2 years, instead of the age of 1 month.

Table 2.1 Additional suitability criteria for paediatric oral dosage forms supplementary to the EMA matrix.

Tablets	A single dose may involve 2 tablets at the maximum
	A single dose may involve a halved tablet, if 1) the tablet contains a score line 2) the SPC does not state that the scoring line is for esthetical reasons only; 3) the SPC does not state that the tablet may only be broken to facilitate the intake of the full dose.
Oral liquid preparations	The maximum dosing volume is 5 mL for children aged below 5 years
	The maximum dosing is 10 mL for children aged from 5 to 10 years
	The minimum single dosing is 0.2 mL

3. Excipients in paediatric formulations

To identify liquid drug products that are unsuitable due to the presence of potentially harmful excipients, four commonly used excipients with known risks were selected; ethanol, propylene glycol (PG), benzyl alcohol and propyl paraben. Limits for safe exposure (maximum daily doses which are considered to be safe) were retrieved from EMA publications and are summarized in Table 2.2. As there are no weight-based limits published for ethanol, we interpreted the single dose limits from the current draft EMA document on ethanol as daily limit (19). To quantify the exposure of our patients to potentially harmful excipients, we studied the actual dosages and drug formulations administered at the paediatric wards, also including parenteral and rectal formulations. Information on the composition of the formulations was retrieved from the SmPC or via direct communication with the marketing authorisation holder or manufacturer. The dataset for the analysis contained all ongoing medication orders for each single day in February 2017 and was obtained from the electronic prescribing systems of the Erasmus MC - Sophia Children's Hospital. The daily administered amounts of excipients were calculated for each individual patient and compared with the recommended values for safe exposure. If patients were on multiple medicines simultaneously, this was factored into the daily exposure calculation. After identifying patients with potentially harmful exposure, we calculated the median (range) exposure per product and age group.

Table 2.2 Excipients and recommended values for safe exposure per age group, derived from EMA publications.

Excipient	Age	Limit
Ethanol (19)	< 2 years	Avoid
	2-5 years	6 mg kg^{-1} day^{-1}
	≥ 6 years	75 mg kg^{-1} day^{-1}
Propylene glycol (23)	Neonates	1 mg kg^{-1} day^{-1}
	1 month – 4 years	50 mg kg^{-1} day^{-1}
	≥5 years	500 mg kg^{-1} day^{-1}
Benzyl alcohol (26)	Preterms and neonates	Not permitted
	4 weeks – 3 years	90 mg kg^{-1} day^{-1}
Propyl paraben (27)	Any	2 mg kg^{-1} day^{-1}

Data analysis

Descriptive statistics were performed with Microsoft Excel 2010.

Table 2.3 Most frequently used compounded drugs accros paediatric hospitals in the Netherlands.

Drug	Therapeutic class according to EMA needs for paediatric medicines	Formulation requirement according to EMA needs for paediatric medicines
Acetazolamide	Neurology	No
Amlodipine	Nephro-urology	Yes
Caffeine	Respiratory	No
Carvedilol	Cardiovascular	Yes
Chloral hydrate	Neurology/Psychiatry	Yes
Clobazam	Neurology	No
Clonidine	Cardiovascular	Yes
Dexamethasone	Endocrinology	No
Enalapril	Nephro-urology	Yes
Furosemide	Nephro-urology	No
Hydrochlorothiazide	Nephro-urology	Yes
Hydrocortisone	Endocrinology/Immunology	Yes
Labetalol	Cardiovascular	No
Lorazepam	Neurology/Psychiatry	Yes
Methadone	Pain	No
Midazolam	Anaesthesiology/Psychiatry	Yes
Nifedipine	Nephro-urology	Yes
Pancreatine	Gastroenterology	Yes
Phenobarbital	Neurology	Yes
Phenytoin	Neurology	No
Prednisolone	Rheumatology/Immunology	Yes
Propranolol	Cardiovascular	No
Sildenafil	Cardiovascular	No
Sodium benzoate	Metabolic dissorders	No
Sotalol (hydrochloride)	Cardiovascular	Yes
Spironolactone	Nephro-urology	No
Tacrolimus	Immunology	No
Topiramate	Neurology/Psychiatry	Yes

RESULTS

1. Availability of paediatric medicines in the Netherlands

Out of the 11 academic and teaching paediatric hospitals that were approached , eight responded and filled out the questionnaire. The survey revealed that for 129 out of 139 drug substances (93%), at least one of the eight respondents stated that a compounded product was needed to meet the needs of their paediatric patient. Table 2.3 displays all medicines for which at least five respondents stated to use a compounded drug. For 13 of these 28 drugs (46%), the EMA inventory of paediatric needs does not state the need for an age-appropriate formulation.

Dispensing of commercial products from the inpatient pharmacy

Over the 10-week study period during the autumn of 2016, 2,274 oral medication orders were evaluated for a total of 437 patients. Our data show that the use of commercially available drugs was lowest in preterm neonates (193/418 prescriptions, 46%) and neonates at the PICU (33/80 prescriptions, 41%) and non-ICU wards (20/54 prescriptions, 37%). Figure 2.1 displays the percentage of commercial products dispensed per age group, for ICU and non-ICU patients.

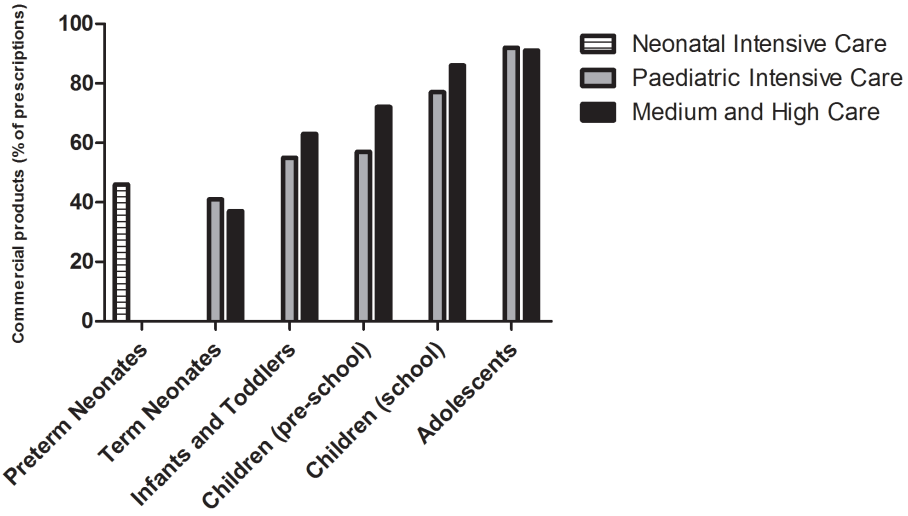

Figure 2.1 Prevalence of dispensing of commercial oral drug products per age category.

2. Age-appropriateness of paediatric formulations dispensed from the inpatient pharmacy

Results from the dose-capability and age-appropriateness assessment depicted in Figure 2.2 revealed that only 402/601 (67%) of dispensed oral formulations for the PICU were considered suitable for the patient according to the set criteria. For the non-ICU wards

this number was higher, with 1047/1255 (83%) dispensed oral formulations regarded as suitable. For the NICU, all 418 dispensed oral formulations were considered unsuitable, as the EMA dosage form matrix considers all oral dosage forms to be unsuitable in preterm new-born infants. Outside of the NICU, dispensing of unsuitable products was most prevalent in neonates and infants at the ICU, with a percentage of 42% in both groups. This was mainly the result from dispensing of solid dosage forms, which are considered unsuitable according to the EMA matrix up to an age of two years. The percentage of dispensed suitable formulations increased with age, up to 94% in adolescent ICU patients, and 88% in adolescent non-ICU patients.

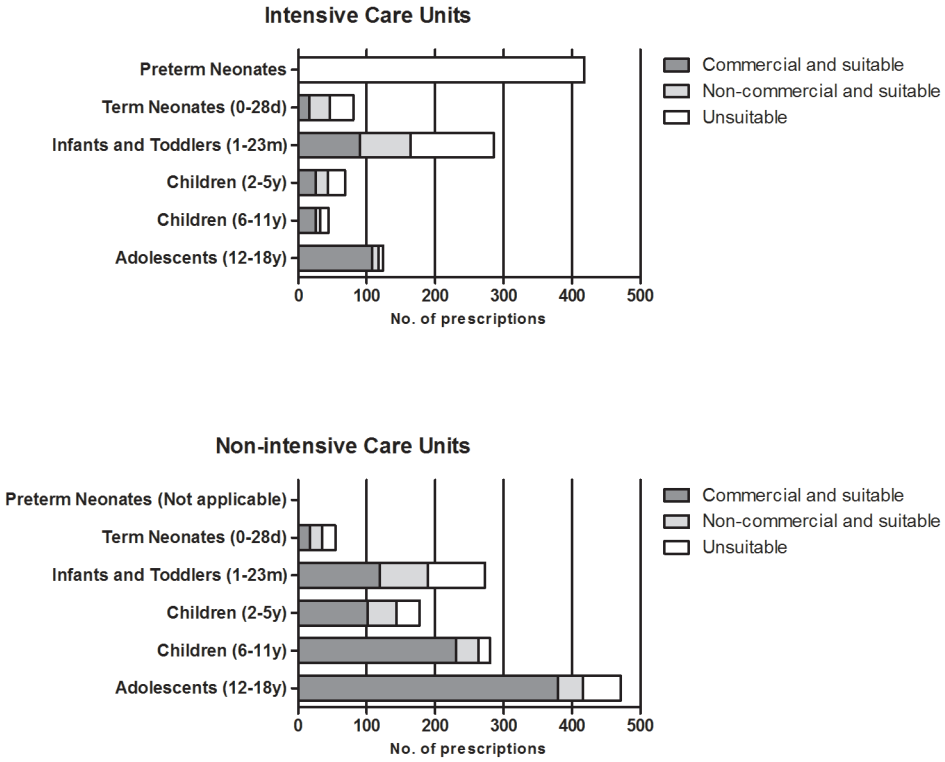

Figure 2.2 Suitability of oral dosage forms dispensed from the inpatient pharmacy.

3. Excipients in paediatric formulations

For the identification of unsuitable drug products due to the presence of potentially harmful excipients, we used a second dataset with prescription data from the inpatient wards of the Sophia Children's Hospital from February 2017. A total of 383 unique patients were admitted and received medication during the study period. From a total of 14,449 medication orders, we identified 40 drug products containing the selected excipients. Safety limits for excipient exposure were surpassed in 22/33 (67%) of NICU patients, 18/77 (23%) of PICU patients, and 16/311 (5%) of non-ICU patients. Exposure sometimes

Table 2.4 Drug products causing excipient exposure above the recommended values for safe exposure. Presented are the number of patients that were exposed to level above the safety limits, and the corresponding median (range) daily exposure per product and age group.

Generic drug name	Brand drug name	Route	Drug conc.	Ethanol conc. (mg mL^{-1})	Age group	No. of patients	Ethanol (mg kg^{-1} day^{-1}) Median and range	
Alprostadil	Prostin VR	IV	0.5 mg mL^{-1}	790	Neonates	7	18.7	14.0-80.6
					Infants and toddlers	2	64.8	14.4-144
Amphotericin B	Fungizone	PO	100 mg mL^{-1}	4.21	Neonates	3	0.9	0.8-1.0
					Infants and toddlers	6	0.6	0.4-2.1
Clemastine	Tavegyl	IV	1 mg mL^{-1}	70	Infants and toddlers	3	6.3	6.2-6.4
Diazepam	Rectiole	Rectal	2 mg mL^{-1}	100	Infants and toddlers	2	1.8	1.8-1.9
Digoxin	Lanoxin PG Elixer	PO	0.05 mg mL^{-1}	81.7	Infants and toddlers	1	5.4	
Nystatin	Labaz	PO	100,000 E mL^{-1}	7.9	Preterms	7	23.8	9.7-48.9
					Infants and toddlers	3	7.8	1.9-11.8
				Propylene glycol conc. (mg mL^{-1})			**Propylene glycol (mg kg^{-1} day^{-1}) Median and range**	
Caffeine	Non-commercial liquid	PO	10 mg mL^{-1}	9.1	Preterms	16	4.8	4.3-9.2
					Neonates	4	4.6	3.3-9.5
Diclofenac	Generic	IV	25 mg mL^{-1}	200	Infants and toddlers	3	72.8	70.6-81.0
Furosemide	Non-commercial liquid	PO	2 mg mL^{-1}	9.1	Preterms	4	4.8	3.9-5.6
					Neonates	7	13.1	5.7-26.4
Hydrochlorothiazide	Non-commercial liquid	PO	0.5 mg mL^{-1}	9.1	Preterms	4	8.0	6.9-16.4
Itraconazole	Trisporal liquid	PO	10 mg mL^{-1}	103.6	Children	1	52.1	
Lorazepam	Temesta	IV	4 mg mL^{-1}	823	Infants and toddlers	2	82.3	64.6-123.5
Potassium chloride	Non-commercial liquid	PO	1 mmol mL^{-1}	6.1	Neonates	1	13.3	
Propranolol	Non-commercial liquid	PO	1 mg mL^{-1}	2.275	Preterms	1	4.4	2.5-5.1
				Propyl paraben conc. (mg mL^{-1})			**Propyl paraben (mg kg^{-1} day^{-1}) Median and range**	
Paracetamol	DARO liquid	PO	24 mg mL^{-1}	0.56	Infants and toddlers	3	2.1	2.0 – 2.1
					Children	1	2.1	2.0 – 2.1

continued over multiple days (median 6, range 1-15 days), and was most frequent with the use of caffeine oral liquid (16 patients, PG), nystatin suspension (10 patients, ethanol) and alprostadil infusion (9 patients, ethanol), which are all administered for prolonged periods if necessary. For propylene glycol, the highest daily exposure was observed for diclofenac IV, lorazepam IV, and itraconazole oral liquid.

In total, we identified 15 products that caused excipient exposure above the recommended values, as displayed in Table 2.4. Five of these products were pharmacy compounded, non-commercial liquids. Propranolol, furosemide and hydrochlorothiazide liquids were prepared according to the Formulary of Dutch Pharmacists (20). Propylene glycol in these products comes from a concentrated methyl paraben solution (15% m/v), used to process the preservative. No benzyl alcohol administration above the safety limit was observed during our study period.

DISCUSSION

The results from this study show that ten years after the introduction of the Paediatric Regulation, there is still a large need for age-appropriate formulations in daily clinical practice. The largest need was observed for the youngest age groups from preterm neonates to infants and toddlers, and the need was higher at ICU wards compared to non-ICU wards.

The widespread use of pharmacy compounded products confirms that the currently available commercial products do not meet the needs of paediatric patients. Almost half of the commonly used compounded products in the Netherlands were not included in the EMA inventory of paediatric needs. Possible explanations are differences in availability between EU countries (for instance phenytoin oral suspension), or need based upon specific indications, but it is not completely clear how the inventory was established.

As mentioned in the introduction, individual compounding carries risks for the patient. In the Netherlands, to mitigate these risks, the Formulary of Dutch Pharmacists aims to standardise compounding and increase the quality. This formulary contains over 160 standardised monographs for extemporaneous formulations, and for each product, quality and shelf-life data are available. Many of these unauthorised products are produced under GMP-conditions in large compounding pharmacies, to obtain medicines of high pharmaceutical quality. On a European level, The European Directorate for the Quality of Medicines & HealthCare (EDQM) has commenced to generate a pan-European paediatric formulary, to improve access to suitable and age-appropriate formulations. This formulary will contain monographs of extemporaneous formulations based on the best approaches currently available in national or regional formularies within Europe (21).

Analysis of our own dispensing data showed that (preterm) neonates and infants were most likely to receive non-commercial, compounded formulations. This can be expected as older children are more likely to be able to receive the correct dose using (manipulated) adult dosage forms. However, the dispensing of a commercial product does not mean that the dosage form is suitable for the patient. When comparing our results to the results of Van Riet-Nales *et al,* who conducted their research seven years earlier and from a regulatory perspective, the percentage of authorised and dose-capable medicines with an age-appropriate formulation was very similar. With our study, these results can now be confirmed from a clinical perspective.

In the assessment of excipient exposure from liquid products, we found that possible toxic exposure was not limited to only NICU patients, but was relevant in children up to the age of four years.

Whittaker *et al* (15) observed ethanol exposure in preterm infants up to 1,8 mL of ethanol per week (1422 mg), uncorrected for weight. In our NICU population ethanol exposure was mainly caused by nystatin treatment, which has a standard dosing schedule of 1mL four times daily, leading to a cumulative exposure of 0,28 mL of ethanol per week (221 mg), which is significantly lower. A follow-up study by the same group found that ethanol concentration in neonates were not elevated after exposure through medication, but they did find elevated levels of acetaldehyde (16). This supports the concept that neonates have minimal systemic exposure to ethanol after enteral administration at the studied dose levels, due to a first-pass effects, but exposure to acetaldehyde might be just as relevant. At the PICU, alprostadil infusions led to ethanol exposure as high as 0,18 mL kg^{-1} day^{-1}in infants and toddlers, which is equivalent to 1 (NL) unit of alcohol for a 70 kg adult. The fact that it is administered intravenously, also means that there is no first-pass effect to decrease the systemic exposure.

The levels of propylene glycol exposure we observed in our population were relatively low compared to the exposure reported by Whittaker *et al.* In preterms and neonates, they did not exceed the WHO acceptable daily intake limit of 25 mg kg^{-1} (15), but often exceeded the EMA limit in neonates of 1 mg kg^{-1} day^{-1}. In infants, toddlers and children, we identified three products that produced significant exposure; diclofenac and lorazepam IV fluid, and itraconazole liquid. Especially the latter is concerning, as treatment often continues over several months, and a therapeutic alternative is not available.

Compared to the results reported by Akinmboni *et al* (22), excipient exposure in our NICU patients was lower (67% vs 98%) compared to exposure in their study population of 106 low birth weight preterm neonates. It is notable that they observed eight different products containing benzyl alcohol, albeit over a study period of a full year, opposed to zero products in our one month study period. In total, they identified 19 products containing unwanted excipients at the NICU alone, compared to only five in our NICU population. This difference can be explained by substitution of unfavorable products with pharmacy compounded alternatives, free of unwanted excipients.

Overall, excipient exposure in our patients was lower compared to other studies. This is probably the result of the ample availability of pharmacy compounded alternatives with regard for suitable excipients. Nevertheless, we identified non-essential products that we should either try to avoid or substitute, and essential medicines in need of improvement. On the other hand, it is important to note that the limits presented by the EMA are actually thresholds, above which it is necessary to provide certain information in the package leaflet. It should be kept in mind that higher doses may be administered when justified (23). The suitability assessment in this study focused on four commonly used solvents and preservatives, but there are more excipients with reports of possible toxicity in paediatric patients, including sweeteners, solubilising agents, and flavourings (24, 25).

Strengths and limitations

The major strength of this study was the use of clinical dispensing data, which enabled the identification of relevant needs in different age groups and level of care settings. Also,

we included the entire age range of paediatric patients in our research. The suitability assessment revealed that at least one-third of dispensed oral dosage forms for the PICU and one-sixth of non-ICU oral medication were not age-appropriate. These results must be interpreted with caution, as the acceptability matrix from the EMA reflection paper was based on sparse evidence. If more recent evidence on acceptability of mini-tablets and multiparticulate dosage forms would have been included in the matrix, the results might have differed slightly. Also, other aspects that might decrease the ability of patients to take solid dosage forms, such as sedation and/or tube feeding, were not considered. Palatability, which is an important component of acceptability, was not considered in the assessment, as it is unknown for most drugs. Future research should focus on generating evidence on patient preference and acceptability of dosage forms, to further assist the development of suitable paediatric drug products. Data collection took place during a specific time of the year, which means that we could have missed some medications that are seasonally dependent.

CONCLUSION

This study confirms there is still a large need for age-appropriate formulations in daily clinical practice, despite the successes of the Paediatric Regulation. The paediatric use marketing authorisation does not provide enough incentive for pharmaceutical corporations to invest in the development of off-patent paediatric drugs. Consequently, pharmacy compounding for paediatric patients remains essential for many indications, and the EDQM paediatric formulary is therefore warranted. Concomitantly, efforts should be made to reduce the exposure to potentially harmful excipients, by avoiding or substituting non-essential medicines, and improving the composition of essential medicines.

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APPENDIX 1

List of drug substances included in the paediatric compounding survey across Dutch paediatric hospitals

Acenocoumarol	Dexamethasone	L-Arginine
Acetazolamide	Dexamphetamine	L-Citrulline
Acetylsalicylic acid	Diazepam	Levodopa + carbidopa 10: 1
Alimemazine	Diazoxide	Levofloxacin
Allopurinol	Diclofenac	Linezolid
Amiodarone (hydrochloride)	Disodium hydrogen phosphate	Lisinopril
Amlodipine	Doxapram	Lorazepam
Ammonium chloride	Enalapril	Magnesium chloride
Aripiprazole	Esketamine	Magnesium citrate
Ascorbic acid	Esomeprazole	Mefloquine
Atenolol	Ethambutol	Melatonin
Azathioprine	Etoposide	Mercaptoethane sulfonic acid
Baclofen	Fenobarbital	Mercaptopurine
Biotin	Fenprocoumon	Methadone (hydrochloride)
Biperiden	Ferrochloride	Methotrexate
Bosentan	Flecainide (acetate)	Metoprolol
Bumetanide	Fludrocortisone	Metoprolol (tartrate)
Calcitriol	Folic acid	Midazolam
Calcium acetate	Folinic acid	Naproxen
Captopril	Furosemide	Nifedipine
Carglumic acid	Gabapentin	Nilotinib
Carvedilol	Glycopyrronium	Nitrazepam
Chloral hydrate	Granisetron	Nitrofurantoin
Chloroquine	Hydrochlorothiazide	Ofloxacin (hydrochloride)
Chlortalidon	Hydrocortisone	Omeprazole
Clobazam	Hydroxychloroquine	Penicillamine
Clonidine	Imatinib	Perampanel
Codeine	Indomethacin	Phenytoin
Coffeine	Isoniazid	Phytomenadione
Colestyramine	Isosorbide	Potassium chloride
Cyclophosphamide	Labetalol	Potassium citrate
Dantrolene orally	Lamotrigine	Potassium iodide

Potassium-sodium phosphate
Prednisolone
Procarbazine
Propafenon
Propranolol
Propylthiouracil
Pyrazinamide
Pyridoxine
Pyrimethamine
Ranitidine
Retinol
Ribavirin
Riboflavin
Sevelamer
Sildenafil
Simvastatin
Sodium benzoate
Sodium chloride
Sodium selenite
Sodium sodium bicarbonate
Sotalol (hydrochloride)
Spironolactone
Sulfadiazine
Sulfasalazine
Tacrolimus
Temazepam
Tetrahydrobiopterin
Theophylline
Thiamazole
Thiamine
Tioguanine
Tiopronine
Tocopherol acetate DL-alpha
Tolterodine
Topiramate
Tranexamic acid
Triamtereen
Trimethoprim
Ursodeoxycholic acid
Valaciclovir
Valganciclovir
Vancomycin
Zonisamide

3

Manipulation of oral medication for children by parents and nurses is common practice and requires proactive instructions from the pharmacy

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ABSTRACT

Aim

Due to a lack of age-appropriate formulations, administration of drugs to children remains a challenge. This study aimed to identify the problems experienced in both the outpatient setting, as well as the clinical setting.

Methods

We performed a cross-sectional, prospective study at the Sophia Children's Hospital, The Netherlands. The study comprised of a structured interview on drug manipulations with parents visiting the outpatient clinic, and an observational study of drug manipulations by nurses at the paediatric wards.

Results

A total of 201 questionnaires were collected from parents/caregivers, accounting for 571 drugs and 169 manipulations (29.6%). Drug substances that were most often mentioned as manipulated were macrogol (n=23), esomeprazole (n=15), paracetamol (n=8), methylphenidate (n=7) and melatonin (n=7). Of all manipulated medicines, 93/169 (55%) were manipulated according to the instructions or recommendations of the SmPC or PIL. Many respondents indicated to have received information on manipulation, but only half of them received this information from their pharmacy. During the observational study, manipulation was performed by 21/35 of observed nurses (60%), of whom 11 deviated from the hospital protocol for manipulation or SmPC (52.3%).

Conclusion

This study showed that manipulation is still a widely used method to administer drugs to children. Validated information regarding manipulation of drugs for both parents/caregivers and nursing staff is needed.

INTRODUCTION

Administration of drugs to paediatric patients remains a challenge for both parents/caregivers and healthcare professionals. The lack of age-appropriate pharmaceutical preparations for children, primarily with respect to accuracy of dosage and routes of administration, contributes mainly to this barrier (1, 2).

Van Riet-Nales *et al.* (2009) showed that only 48% of available medicines for human use were authorised for one or more paediatric age groups (1), and the recent 10-year report of the Paediatric Regulation confirms the lack of progress for off-patent medicines (3). Furthermore, a paediatric indication in the label does not necessarily mean that the dosage from is suitable for use in children (1). The inventory of needs for paediatric medicines from the European Medicines Agency (EMA) still shows there is a lack of age-appropriate paediatric products in a considerable number of therapeutic areas (4). This lack of age-appropriate formulations forces parents and caregivers to apply manipulation techniques to the medicine in order to achieve the appropriate dose or to make the medicine acceptable to their children (2). In the clinical setting, manipulation also occurs frequently, either within the pharmacy in the preparation of extemporaneous formulations, or in the wards at the moment of administration (5).

There are risks attached to the manipulation of medicines. In a recent review, Richey *et al.* (6) summarized the evidence for the use of dosage form manipulation to obtain the required dose. Multiple researchers showed that splitting tablets by hand, with a kitchen knife or even a tablet splitter caused inconsistent results in terms of dose accuracy (7-12). Dispersing tablets in water and taking a portion of the obtained suspension is another method to adjust the dose. However, doses may vary depending on where the samples are taken from the container used to disperse the drug, especially for poorly water-soluble drugs (13). Moreover, drug loss during manipulation can be a significant problem, depending on the drug, operator and method used (6).

Besides the possible negative effects on dose accuracy, accompanying risks of manipulation include possible negative effects on stability, solubility and bioavailability, with subtherapeutic or even toxic drug levels as an unwanted result (14, 15). Lastly, manipulations are time-consuming and could increase the risk of errors, given the fact that drug calculation errors are the most common type of errors in neonatal and paediatric practice (2). Therefore, there is a need to standardise procedures to reduce the risks associated with manipulation. In the Netherlands, a reference work for manipulation of drugs,'Oralia VTGM', is issued by *The Royal Dutch Pharmacists Association*, and available via subscription.

In summary, various studies showed the risks of drug manipulation, induced by the lack of authorised and age-appropriate paediatric medicines, but few studies have evaluated the extent and type of manipulation. The aim of this study was to identify the problems in the administration of drugs to children experienced by both parents/caregivers in the outpatient setting, as well as by nurses in the clinical setting, by determining the extent, reasons and methods used for drug manipulation.

In order to achieve this aim, we made use of 1) a questionnaire to determine the methods and tools used by parents/caregivers and 2) observations of drug administrations to paediatric patients by nurses to determine the frequency and types of manipulations.

A secondary objective was to identify the information sources parents/caregivers and nurses use to execute manipulation, in order to identify gaps in the availability of instructions.

METHODS

Study design

We performed a cross-sectional, prospective study at the Erasmus MC—Sophia Children's Hospital, a tertiary referral hospital in Rotterdam, The Netherlands, between July 2017 and January 2018. The study consisted of two parts. First, we conducted a survey on drug manipulations by parents/caregivers of outpatients. Second, we conducted a structured, undisguised, observational study of drug manipulations by nurses at the paediatric wards. For the purpose of this study, manipulation was defined as 'the physical alteration of a pharmaceutical drug dosage form for the purpose of extracting and administering the required proportion of the drug dose'. This definition is based on the Manipulation Of Drugs In Children (MODRIC) guidelines from the Alder Hey Children's Hospital (16). In addition, drugs co-administered with food or liquids that are not explicitly recommended, without physical alteration of the dosage form, were also accounted as manipulation.

Manipulations by parents/caregivers in the outpatient setting

Questionnaire

An electronic questionnaire was built using the web-based LimeSurvey version 2.06 (LimeSurvey GmbH, Hamburg, Germany). The questions were derived from sources regarding the manipulation of medicines for paediatric administration (MODRIC guidelines), and additional research regarding manipulation of medication in children (2, 6, 17-20). Questions gave insight into the extent, reasons, and methods of manipulation of oral dosage forms for children by parents and caregivers, and included six topics; demographic data, current medication, methods and reasons for manipulation, medication adherence in relation to manipulation, the possible combined administration of oral medicines, and the sources of information consulted regarding manipulation. Before start of data collection, the questionnaire was reviewed by pharmacy-technicians from the outpatient pharmacy of the Erasmus MC, to test the length of the questionnaire and the comprehensibility of the questions for parents and children. After processing the feedback from the pharmacy-technicians, the questionnaire was piloted using 20 participants to resolve any remaining ambiguities in the questions.

Recruitment

Participants were recruited at the outpatient clinics representing all major paediatric subspecialties, and before and after the medication reconciliation visits related to hospital admission. Inclusion criteria were the use of oral medication and age below 18 years. Insufficient command of the Dutch language was an exclusion criterion. The questionnaire was filled in by the researcher whilst interviewing the parent/caregiver and/or patient. With permission from the participants, we verified the answers regarding current medication with their outpatient medication list retrieved from the outpatient pharmacy or their local pharmacy.

Manipulations by nurses at the inpatient wards

To assess the extent and ways of drug manipulation by nurses, the researcher observed the administration of oral medication to paediatric patients. Nurses were informed of the intention of the study: to improve drug therapy in patients, and not to assess any individual performances. Observation of paediatric nurses took place for one week in each of the six wards (Paediatric intensive care unit, Neonatal intensive care unit, Oncology, Neurology/Neurosurgery, General paediatrics, Paediatric surgery/Paediatric Thorax centre). A minimum of five nurses were observed at each ward.

Data analysis

After collection of the data, the manipulations were compared to the patient information leaflet (PIL) (parents/caregivers), the summary of product characteristic (SmPC, parents/caregivers and nurses), or the local hospital protocol for drug manipulation and administration (nurses), to check if they were performed according to any of the instructions.

Age categories were defined according to the guideline on clinical investigation of medicinal products in the paediatric population (21), and all patients admitted to the NICU were categorised as preterm neonates.

Ethical approval

The Erasmus MC Medical Ethics Committee reviewed the research proposals of both study parts, and decided that they did not fall within the scope of the Medical Research Involving Human Subjects Act (ref no. 2017-276 and 2017-1092). Nevertheless, participants in the questionnaire were asked for written consent.

RESULTS

Manipulations by parents/caregivers in the outpatient setting

Between June 2017 and January 2018, a total of 201 questionnaires were collected from parents/caregivers visiting the outpatient clinics of the Sophia Children's Hospital. The total number of oral medicines reported was 571. Patient characteristics are displayed in Table 3.1.

Table 3.1 Patient characteristics.

	N	Median (IQR)	%
Age			
Term neonates (0d-28d)	0		0.0 %
Infants and toddlers (1m-23m)	25	0.8 (0.3-1.5)	12.4 %
Children, pre-school (2y-5y)	72	3.8 (3.0-4.6)	35.8 %
Children, school (6y-11y)	63	8.0 (7.0-11.0)	31.3 %
Adolescent (12y-17y)	41	14.0 (12.7-15.5)	20.4 %
Total	**201**	**6.0 (3.3-11.0)**	**100 %**
Sex			
Male	113		56.2 %
Female	88		43.8 %
Presence of feeding tube			
Nasogastric tube	10		
Percutaneous endoscopic gastrostomy	22		
Total	32		15.9 %

d = days, m = months, y = years

Methods and reasons for manipulation

The survey revealed that 106/201 (53%) respondents applied manipulation to one or more drugs before administration. In total, 169/571 (29.6 %) medicines were manipulated. Drug substances that were most often mentioned as manipulated were macrogol (n=23), esomeprazole (n=15), paracetamol (n=8), methylphenidate (n=7) and melatonin (n=7). Figure 3.1 displays the reasons for manipulation, divided per age group, with taste mentioned as main reasons for manipulation, followed by dose adjustment. Figure 3.2 displays the methods for manipulation, with mixing with a liquid mentioned most frequently, followed by breaking or splitting of a tablet.

Of all manipulated medicines, 93/169 (55%) were manipulated according to the instructions or recommendations of the SmPC or PIL and 69/169 (41%) were manipulated not fully according to the SmPC or PIL. For 7/169 manipulated medicines, which were extemporaneously compounded, no SmPC or PIL was available. Table 3.2 provides an overview of the types of manipulation, deviating from the SmPC or PIL. For seven of the

manipulated medicines no SmPC or PIL was available.

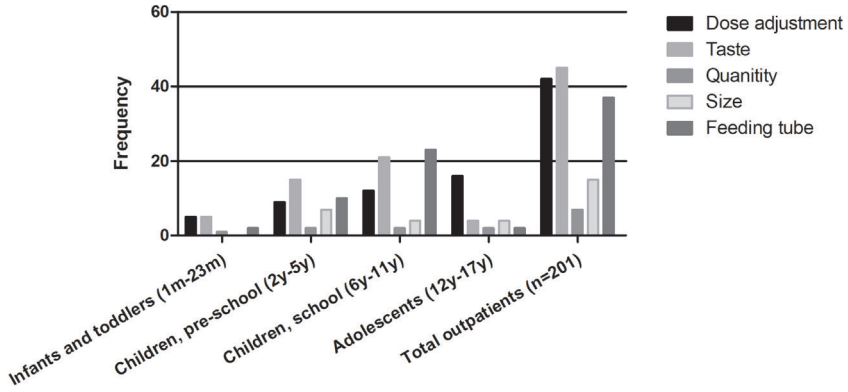

Figure 3.1 Reasons for manipulation of oral dosage forms (n=169) reported by parents/caregivers.

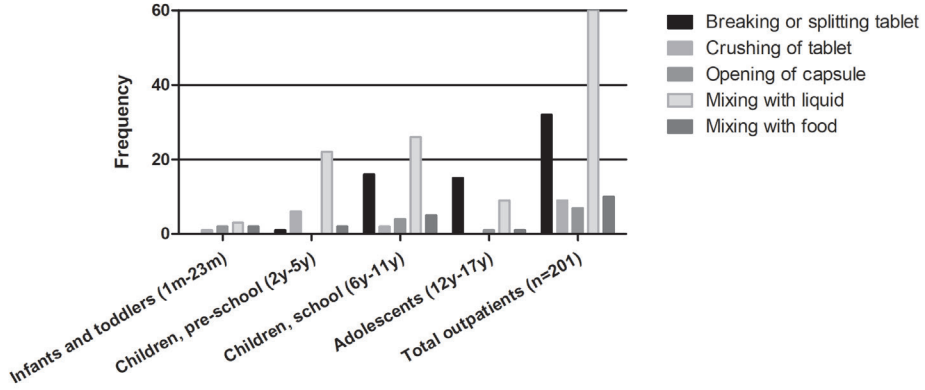

Figure 3.2 Methods for manipulation of oral dosage forms (n=169) reported by parents/caregivers.

Table 3.2 Classification of manipulation not according to the SmPC, with a definition of the type of manipulation.

Type	Definition	Frequency	% (n=69)
Vehicle	Use of food or drink to aid administration other than what is recommended in the SmPC/PIL	36	52.2%
Dose	Manipulation that might not provide an accurate dose (e.g. splitting of unscored tablets)	22	31.9%
Integrity	Affecting or breaking the integrity (e.g. coating) of a drug by manipulation	8	11.6%
Mixing	Administration of multiple medicines by mixing them (e.g. by adding the content of a capsule to a syringe with a liquid drug)	6	8.7%
Safety	Manipulations that cause a risk for the parent/ caregiver (e.g. crushing of methotrexate)	2	2.9%

SmPC = summary of product characteristics, PIL = patient information leaflet

Information sources

All respondents who replied to perform some form of manipulation were asked if they had received information on manipulation, or acquired it themselves from any source. As displayed in Table 3.3, 45% of the respondents reported to have received explicit information on manipulation, and 13% of the respondents indicated to not have received any information when it might have been applicable. Verbal information was more common than written information and the pharmacy was the most frequently cited source of information.

Table 3.3 Information source used by parents/caregivers for drug manipulation.

Instructions provided for manipulation	Frequency	Percentage of total (n=116)
Yes	90	78%
No	26	22%
Not applicable	85	
Type of communication		
Verbal	46	40%
Written	11	9.5%
Both	33	28%
Source of information		
Doctor	35	30%
Nurse	28	24%
Pharmacist or pharmacy technician	44	38%
Patient Information Leaflet	20	17%
Internet	0	0%
Other	1	<1%

Manipulations by nurses at the inpatient wards

Observations of nurses at the wards took place during a study period of six weeks, and within this period 115 drug administrations to 35 individual patients were observed, performed by 35 different nurses. Patient characteristics and qualification of the nurses are displayed in Table 3.4.

Reasons and methods for manipulation

Manipulation of the dosage form was required for 21/35 observed patients (60%). Also, manipulation was performed prior to 42/115 oral administrations (36.5%). The frequencies of the performed methods for manipulation and the corresponding reasons are displayed in Figure 3.3. Drug manipulations prior to administration were compared to the instructions from the SmPC and the hospital protocol. Of the 42 observed manipulations, 26 (62%) were given conform SmPC or hospital protocol and 16 (38%) deviated.

Table 3.4 Patient/nurse characteristics.

Qualification nurses	Frequency	Percentage of total (n=35)
Student nurse	2/35	5.7%
Registered nurse	8/35	22.9%
Advanced practice registered nurse	25/35	71.4%
Patient age category	**Frequency**	**Percentage of total (n=35)**
Preterm neonates (NICU)	4	11.4%
Term neonates (0d-28d)	2	5.7%
Infants and toddlers (1m-23m)	9	25.7%
Children, pre-school (2y-5y)	7	20.0%
Children, school (6y-11y)	5	14.3%
Adolescent (12y-17y)	8	22.9%
Sex (n)		
Male	21	60%
Female	14	40%
Use of feeding tube (n)	17	49%

d = days, m = months, y = years

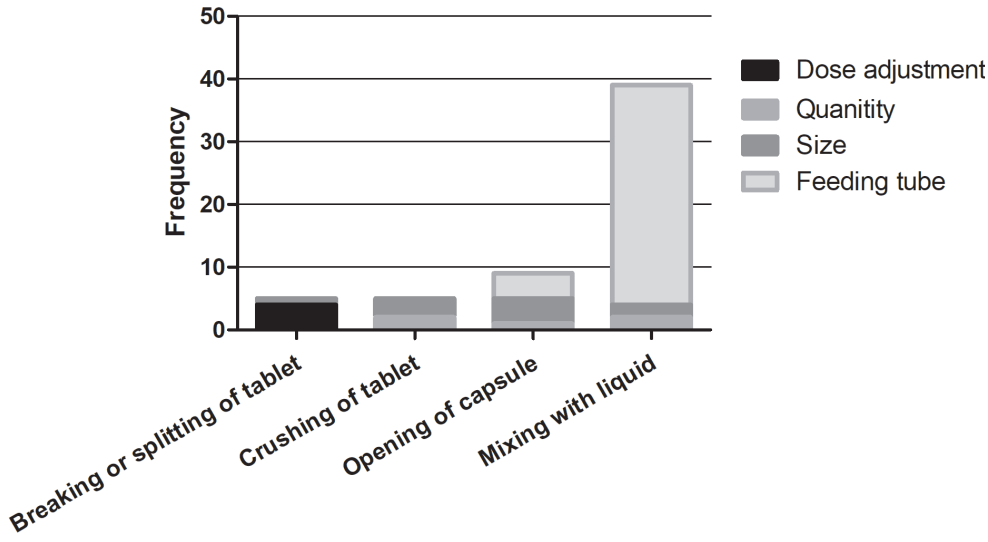

Figure 3.3 Observed methods of manipulation with corresponding reasons reported by nurses for 42 drug administrations.

Information sources

Manipulation was performed by 21/35 nurses (60%), of whom 11 deviated from the hospital protocol for manipulation or SmPC (52.3%). Most often these deviations consisted of opening of capsules to mix the content with liquid, grinding of tablets with the risk of drug loss, and mixing drugs with incompatible food or liquids (e.g. dairy). Nurses that performed manipulations were asked about any instructions that they received and information sources they consulted regarding the manipulation (Table 3.5).

Table 3.5 Information source consulted by nurses for drug manipulation.

Instructions provided for manipulation	Frequency	Percentage of total (%)
Yes	16/21	76.2%
No	5/21	23.8%
Type of communication		
Written	6/16	37.5%
Verbal	9/16	56.3%
Both verbal and written	1/16	6.3%
Source of information		
Doctor	1/21	4.8%
Other nurses	10/21	47.7%
Oralia VTGM or hospital protocol	6/21	28.6%
Own knowledge/experience	4/21	19.0%

DISCUSSION

This study showed that manipulation of oral dosage forms is common practice among both parents/caregivers as well as nurses in a paediatric hospital, with a similar prevalence of 30% in the outpatient setting versus 37% in the inpatient setting. Due to a broader definition of manipulation, including the co-administration with possibly incompatible food or liquids, the prevalence in our inpatient cohort was higher compared to the prevalence from a study in two Norwegian paediatric hospitals (37% versus 17%), but the prevalence in our outpatient cohort was equal to a cohort of outpatients from the UK (30% versus 29%) (22).

The predominant reasons for manipulation were different between the inpatient and outpatient setting. Manipulation by parents/caregivers occurred mainly to achieve taste and dose adjustment, whilst nurses most often used manipulation for administration through a feeding tube and size reduction. This difference probably results from the more extensive formulary of the inpatient pharmacy, which allows for more precise dosing with compounded liquids and capsules of different strengths and the higher prevalence of feeding tubes in the inpatient setting.

The predominant method of manipulation, both in the in- and outpatient setting, was

mixing with liquids. In the inpatient setting tube feeding and breast milk were commonly used matrices. When manipulation did not occur according to the instructions, this was most often because of co-administration with liquids or food not mentioned in the SmPC or PIL. Co-administration with liquids or food is often an acceptable strategy to administer drugs to children, but for certain drugs, food-drug interactions can have a significant effect on bioavailability and therapeutic effect (23). Even when such an interaction is known to the nurse, separated administration is not always possible due to administration of enteral feeding. Within our study, this was observed for both ciprofloxacin tablets and itraconazole liquid. Both the reasons and methods used for manipulation by parents/caregivers and nurses were similar to the Norwegian and UK studies, with taste being the most cited barrier to administration in the outpatient setting (17, 22).

Many respondents to the questionnaire indicated to have received information on manipulation, but only half of them received this information from their pharmacy. This is an important finding, as guidance regarding the correct use of medication is one of the main tasks of the pharmacy, and pharmacist have the Oralia VTGM reference book at their disposal. Similarly, only 6/21 nurses stated to have consulted the pharmacy-provided information regarding manipulation of the administered drugs, whilst 38% of the manipulations were not performed according to protocol. It demonstrates the need for additional in-service training of the nursing staff regarding drug manipulation and the available reference works, available through the workstations in the hospital.

In the outpatient setting, taste was an important reason for manipulation, and administration with a vehicle not recommended was the most frequent manipulation not according to the SmPC/PIL. The macrogol containing laxatives were highly represented in this group, as they are very commonly prescribed and the SmPC/PIL recommends only water for administration. In the pharmacy of the Sophia Children's Hospital, the neutrally flavoured products are dispensed and parents are advised to use a fruit syrup to their child's liking to improve the taste. Ideally, the SmPC and PIL should give clear instructions on what food and/or drinks, if any, have been demonstrated to be appropriate for mixing with the paediatric preparation, as is now part of the Guideline on pharmaceutical development of medicines for paediatric use (21). Unfortunately, this information is not available for a lot of medicines, and recommendations are made on the basis of physical-chemical formulation and drug characteristics. The absence of standard methods or criteria that define what flavours are acceptable to children, and the absence of common vehicles which are widely accepted and available, complicate the compatibility studies needed to form the recommendations regarding the intake with food and liquids (23, 24).

Strengths and limitations

This study took place at a tertiary paediatric hospital, with all the major and minor paediatric subspecialties available, which allowed us to collect a large and diverse dataset. Identification of the difficulties experienced when administering formulations to children is essential for directing future formulation development work. To our knowledge, this is the first study to directly compare the inpatient and outpatient setting with regard to manipulation of oral medicines. The major limitation of this study was the absence of a validated questionnaire and an established definition of manipulation, which limits the comparison of results to other studies. A risk of inaccurate reporting exists with the use of the questionnaire, but missing information regarding current medication was very often retrieved via the patient's local pharmacy or hospital record.

This study was not designed to assess the clinical impact of the reported manipulations. For many drugs, a correctly performed manipulation will not affect the therapeutic performance. However, with every manipulation, there is a risk of error, and complicated manipulations also rely on correct information transfer from the health care professional to the parent/caregiver.

Recommendations

To reduce the need for manipulation, continuous efforts should be made to develop age-appropriate formulations providing both dosing flexibility as well as acceptable taste. Furthermore, as co-administration with food or liquids remains the most practiced strategy for drug administration, more elaborate and explicit information within the SmPC and PIL regarding suitable vehicles is warranted. Dose accuracy remains a problem, especially in the outpatient setting, and efforts should be made to reconcile the inpatient and outpatient formulary, to provide parents/caregivers with more dose-capable formulations. When a patient is discharged, there is an important task for the pharmacist/technician to properly inform parents/caregivers on manipulation and co-administration with food. This also applies to community pharmacies that dispense possibly unsuitable drug products to paediatric patients.

CONCLUSION

This study showed that there remains a need for age-appropriate medicines that can deliver correct dosages, as well as a need for improvement of information regarding manipulation of drugs towards both parents/caregivers and nursing staff.

ACKNOWLEDGEMENT

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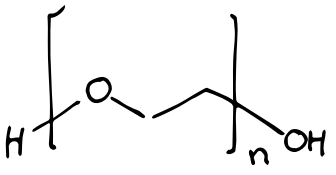
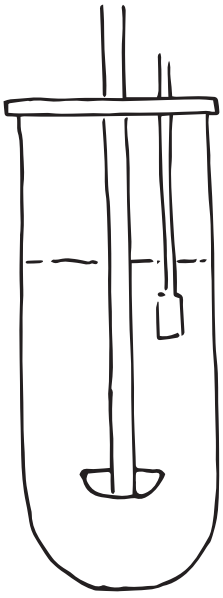
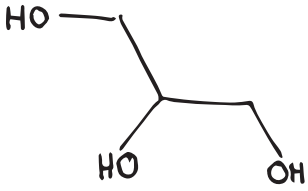
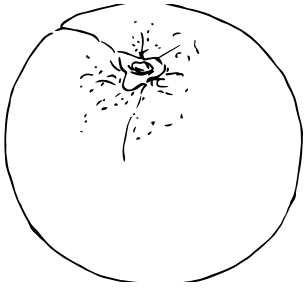
PART II

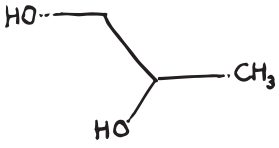
Pharmaceutical development and *in vitro* evaluationof the formulations











4

Design and stability study of an oral solution of amlodipine besylate for pediatric patients

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ABSTRACT

Introduction

Amlodipine is an antihypertensive agent recommended for the management of hypertension in children and adolescents. The commercially available tablets of 5 and 10 mg do not provide the necessary flexibility in dosing needed for treating children. Our goal was to develop a pediatric oral solution of amlodipine, using a robust manufacturing process suitable for ex-tempora and larger scale production.

Methods

The parameters API and preservative content, related substances, appearance and pH were studied under four different storage conditions. Samples were analyzed up to 12 months. Microbiological quality was studied in an 18-week in-use test based on a two-times daily dosing schedule.

Results

The stability of the formulation was influenced by storage conditions and composition. A formulation containing amlodipine besylate, sucrose syrup and methyl paraben remained physically stable for 12 months at 4°C with no loss of amlodipine content. Related substances increased during the study but remained below 0.5%. In-use stability was proven up to 18 weeks.

Discussion

Storage under refrigerated conditions was necessary to prevent precipitation and to obtain an acceptable shelf-life. In conclusion, we have developed and validated an amlodipine oral solution, suitable for the pediatric population. This liquid formulation is preferred over manipulated commercial dosage forms or non-standardized extemporaneously compounded formulations.

INTRODUCTION

Amlodipine (3-O-ethyl 5-O-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate) is a long-acting dihydropyridine L-type calcium channel blocker widely used in both adults and children. It selectively inhibits calcium ion influx in vascular smooth muscle and cardiac muscle, thereby inhibiting the contractile processes of these tissues. The resulting peripheral arterial vasodilation and reduction in peripheral vascular resistance reduces the arterial blood pressure (1). Currently, amlodipine is one of the antihypertensive agents recommended by the European Society of Hypertension for the management of hypertension in children and adolescents (2). Within the group of calcium channel blocking agents, amlodipine is considered first choice treatment for chronic hypertension in children, because of its pharmacological characteristics and it being the most extensively studied drug within this class (2, 3). Calcium channel blockers are also specifically recommended as the preferred drug class in pediatric posttransplantation hypertension (2).

Amlodipine is prescribed off-label to children from the age of 1 month in a dose of 0.06-0.3 mg/kg per day (2). Using the commercially available tablets of 5 and 10 mg, these dosages cannot be administered accurately in young children. Amlodipine has therefor been added to the 'Inventory of paediatric therapeutic needs' published by the European Medicines Agency (EMA), as there is no age-appropriate formulation available (4).

According to the EMA reflection paper on pediatric formulations, an oral liquid dosage form would generally be the form of choice and best applicable to administer systemic medication to infants and toddlers (1m-2y) and young children (2-5y). If the physical and chemical characteristics as well as the taste of the drug substance are appropriate, solutions are preferred over suspensions due to better oral acceptance. In addition, solutions are less susceptible to dosing errors resulting from insufficient re-dispersion and are easier to administer through an enteral feeding tube. Further properties that need to be taken into account when designing a pediatric oral liquid dosage form are dose volume (preferably ≤ 5mL for children under 5 years) and use of child-friendly excipients (5).

Since amlodipine is slightly soluble in water (6, 7) and not prone to chemical degradation when protected from light (8), an oral solution might be a feasible dosage form. Its pharmacokinetic characteristics make a once-daily dosing schedule possible, without the need for a controlled release formulation (9). This aids in compliance and acceptability by pediatric patients (10).

For amlodipine only extemporaneously compounded suspensions have been formulated, using commercially available generic suspension bases (Ora-Plus®/Ora-Sweet® 1:1 and Syrspend® SF) and crushed tablets or amlodipine besylate raw material, resulting in a limited stability of 3 months (8, 11, 12). In The Netherlands, as well as in other EU countries, centralized officinal production of unlicensed medicines by GMP-certified pharmacies is common practice. These products are supplied to other pharmacies, after which they are dispensed to the patient. Simultaneously, a part of the community pharmacies still has compounding facilities to provide ex-tempora formulations to their own patients. This situation requires a formulation design that provides an acceptable shelf-life for batch-production, but at the same time allows for individual ex-tempora compounding.

Our goal was to develop and validate a pediatric oral solution of amlodipine, using a

robust manufacturing process (suitable for extemporaneous compounding). To maximize affordability of and accessibility to the formulation, we chose to make use of manufacturing methods suitable for individual and larger scale production.

MATERIAL AND METHODS

Initially an attempt was made to develop a solution of 1 mg/mL amlodipine besylate, preserved with methyl paraben 0.15% and buffered with citric acid. The aqueous solubility of amlodipine was so low that it required heat to dissolve and precipitated shortly after preparation. Lowering the amount of methyl paraben or citric acid buffer did not improve the stability. The concentration of amlodipine was then lowered to 0.5 mg/mL (equal to 0.69 mg/mL amlodipine besylate), which is an acceptable concentration for application in clinical practice.

Composition

The starting point for the comprehensive development of our formulation was an aqueous solution containing amlodipine besylate. Methyl paraben was maintained as a preservative, since it is considered suitable for use in pediatric formulations (13). Because we aimed for a shelf-life of at least six months, preservative-free formulations were not considered. To enhance the taste of the formulation, sucrose syrup was added until an acceptable taste achieved, according to our experienced formulation developers (Composition A). Additional artificial flavors were omitted to preclude a negative influence on the physical stability. Because of the limited aqueous solubility of amlodipine, a second formulation containing propylene glycol as a co-solvent was studied (composition B). Thirdly, a formulation containing amlodipine maleate (Composition C) was studied, to examine if the aqueous solubility of the maleate form would be better. All formulations were manufactured in batches of 2500 mL (A and B) or 5000 mL (C) and put into 100 mL, amber-colored polyethylene terephthalate (PET) (A, B and C) or glass containers (C). Composition A was prepared with active pharmaceutical ingredient (API) from two suppliers (Duchefa and Wyeth), so four batches of amlodipine solution were manufactured, of which the compositions can be found in Table 4.1. Amlodipine besylate, maleate and all other excipients were European Pharmacopoeia grade.

Long-term stability studies

The influence of temperature, packaging material and amlodipine salt form on long term stability were investigated. Samples were stored in climate cabinets at 4±2°C (Elbanton type 5KV-2-50), 25±2°C (Elbanton type LC 500) and 40±2°C (Elbanton type LTKB-ST650). In each cabinet the temperature was registered hourly. Samples of composition A and C were additionally stored at ambient temperature and indirect daylight. Influence of packaging material was studied on composition C, samples were stored in PET and glass containers under each storing condition. API and preservative content were examined over time. Initially, we aimed for a shelf life of 6 months. Samples were analyzed at 0, 1, 2, 3 and 6 months. With an extension of the stability studies, samples stored at 4°C were subsequently also analyzed at 9 ant 12 months.

Table 4.1 Compositions of the studied formulations, which were manufactured in batches of 2500 mL (A and B) or 5000 mL (C) and put into 100 mL, amber-colored polyethylene terephthalate (PET) (A, B and C) or glass containers (C).

Composition A			
Amlodipine besylate	69	mg	
Methyl paraben solution 15% m/v*	287	mg	
Sucrose syrup^	32	g	
Purified water	75,137	g	
	107,51	g	(=100 mL)
Composition B			
Amlodipine besylate	69	mg	
Methyl paraben solution 15% m/v*	432	mg	
Sucrose syrup^	10	g	
Propylene glycol	3,796	g	
Purified water	88,133	g	
	102,43	g	(=100 mL)
Composition C			
Amlodipine maleate	64	mg	
Methyl paraben solution 15% m/v*	304	mg	
Sucrose syrup^	32	g	
Purified water	75,442	g	
	107,81	g	(=100 mL)

*Methyl paraben is processed as a 15% m/v solution in propylene glycol.
^ Sucrose syrup contains 63% m/v sucrose and 0,1% m/v methyl paraben

The stability indicating HPLC-UV method for determination of API, related substances and preservative content was modified from the Ph. Eur. method of amlodipine besylate drug substance by introduction of a gradient in the mobile phase. Analytical specifications can be found in Table 4.2. Release and end-of-shelf-life specifications are displayed in Table 4.3.

Table 4.2 Analytical specifications of the stability indicating HPLC-UV assay of amlodipine oral solution, derived from the Ph. Eur. monograph of amlodipine besylate.

Column	Spherisorb ODS1, 5 µm, 250 x 4.0 mm		
Test solution	20 µL of 2 ml amlodipine besilate oral liquid in 18 ml water R		
Reference solution	20 µL of 0.05 mg/ml amlodipine as besilate in 1:9 methanol R and water R		
Wavelength	237 nm		
Flow	1.5 ml/min		
Temperature	30°C		
Mobile phase	A: 2.3 g/L ammonium acetate R in water R		
	B: Methanol R		
Gradient	Time (min.)	Solution A (%)	Solution B (%)
	0	50	50
	5	50	50
	6	30	70
	35	30	70
	36	50	50
	45	50	50

In-use stability

An in-use test was performed on Composition A based on a two-times daily dosing schedule. Based on the results from the stability studies, the containers were stored at 4°C and twice-daily removed from the climate chamber to be exposed to air, light and ambient temperature for 30 minutes at every dosing simulation. Samples of 0.4 mL were withdrawn until a quantity of 25 mL remained after which the dosing simulation continued without taking samples. After 18 weeks the samples were analyzed in accordance with the specifications in Table 4.3.

Manufacturing procedure

The amlodipine drug substance was added to ca. 60% of the total volume of distilled water. Using a magnetic stirrer and heating up to 50°C, amlodipine dissolved completely. Methyl paraben solution 15% m/v was added and the mixture was stirred vigorously using the magnetic stirrer. The mixture was cooled to ambient temperature and the sucrose syrup was added. Finally distilled water was added to the solution to reach the desired volume.

Table 4.3 Release and end-of-shelf-life specifications amlodipine besylate solution 0.5 mg/mL. Microbiological tests of the formulation were performed in two samples from the finished in-use stability study.

Test Item	Method	Reference	Acceptance Criteria
Identification	According to assay	Ph. Eur. Amlodipine Monograph	Spectra should be identical to reference
Assay (API and preservative)	HPLC-UV	Modified Ph. Eur. method	90% ≤ content ≤ 110%
Related substances	HPLC-UV	Modified Ph. Eur. method	Total related substances ≤ 1.5%
Appearance	Visual observation	Ph. Eur. 2.2.1	Clarity ≤ O1
		Ph. Eur. 2.2.2	Coloration < GY6
pH	pH meter	Ph. Eur. 2.2.3	Range 5.0 – 6.5
Microbiological quality	Milliflex Plus	In-house procedure	TAMC ≤ 10^2 CFU/mL
	0,45 µm funnel 100 mL		
	TSA Casette		

Ph. Eur. = European Pharmacopoeia; TAMC = Total aerobic microbial count

RESULTS

Stability studies

The physical and chemical stability of the amlodipine solutions were influenced by the storage conditions. At 25°C and 40°C, resulting from both precipitation and chemical degradation, amlodipine content declined over time as displayed in Figure 4.1. Results after six months for Composition A and B are displayed in Table 4.4. A gradual increase in related substances was seen in all samples, but was notably higher with increasing temperatures.

Table 4.4 Results from the stability studies at 6 months.

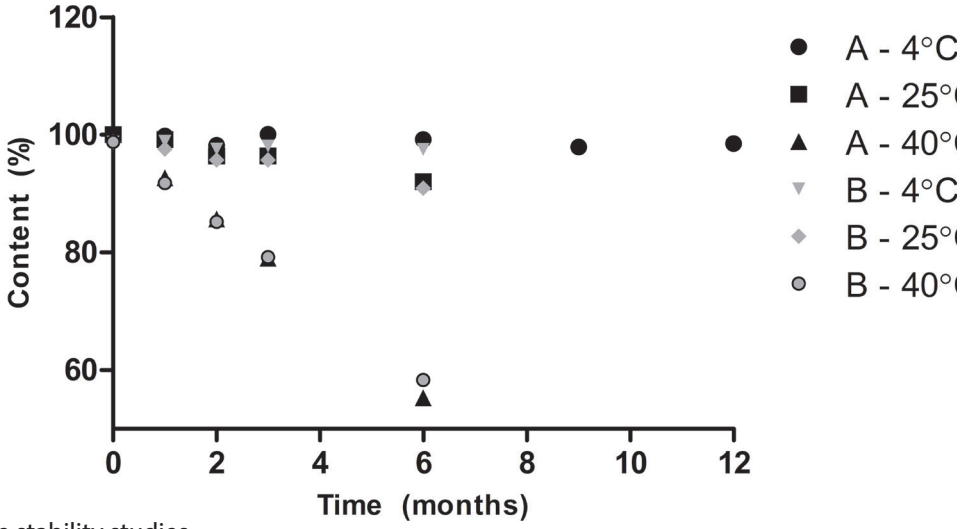
	Storage condition and composition		Appearance			pH	Amlodipine content	Preservative content	Related substances
			Clarity[1]	Color[2]	Particles		(%)	(%)	(%)
T=0		A	≤ O1	<GY6	-	5.9	100.0	95.6	<0.1
		B	≤ O1	<GY6	-	5.7	98.8	95.3	<0.1
T=6	4˚C	A	≤ O1	<GY6	-	5.8	99.2	94.9	<0.1
		B	≤ O1	<GY6	-	5.6	97.5	95.1	0.2
	25˚C	A	≤ O1	<GY6	+	5.6	92.0	95.6	2.1
		B	≤ O1	<GY6	+	5.4	90.9	96.1	1.8
	40˚C	A	< O3	<GY6	+	4.6	55.3	93.7	4.0
		B	< O2	<GY6	+	4.8	58.3	95.3	3.2
	Ambient conditions	A	NA	NA	NA	NA	94.1	95.4	1.5

[1] Refer to Ph. Eur. 2.2.1
[2] Refer to Ph. Eur. 2.2.2.
NA; not available

Based upon the stability results at six months, the stability studies were continued with composition A, since the addition of propylene glycol to Composition B did not provide any advantages. During the extended stability studies, Composition A remained physically stable for 12 months at 4°C with an amlodipine content of 98.7% (see Figure 4.1) and total related substances of 0.5% (not shown). At ambient temperature, the formulation was physically stable for at least two months.

In Composition C particles were first seen after two weeks at 40°C. Crystal depositions were visible in both PET and glass containers. After 3 weeks particles were also seen in Composition C stored at 25°C. The stability studies of Composition C were at this point discontinued.

No changes in color and clarity were observed in any of the samples before precipitation occurred. The methyl paraben content did not decrease in any of the formulations during



the stability studies.

Figure 4.1 Amlodipine besylate content over time for Composition A and B under different storing conditions. After six months stability studies were continued with the preferred composition A.

In-use stability

The samples of Composition A remained physically stable during the in-use study, no crystal depositions of amlodipine were formed. Both the content of amlodipine and methyl paraben increased with 6-8% as a result of evaporation of water. The related substances reached a maximum of 0.2%. The total bacteria count was less than 100 cfu/mL at week 18 of the in-use study in all samples.

DISCUSSION

In this study, we developed an oral solution of amlodipine besylate with adequate physical and chemical stability, a shelf-life of 12 months and excipients suitable for pediatric patients.

The biggest challenge in the development of the formulation was the poor aqueous solubility of amlodipine. The Ph. Eur. describes amlodipine besylate as 'slightly soluble in water', which would mean that it has a solubility of 1 to 10 mg/mL. This is consistent with the solubilities submitted by Pfizer in the US patent (14). In our pre-studies, a stable aqueous solution of 1 mg/mL or higher appeared not to be feasible, therefore we reduced the concentration to 0.5 mg/mL. Storage under refrigerated conditions was necessary to prevent precipitation and to obtain an acceptable shelf-life. With precipitation occurring faster at higher temperatures, it appears to be an endothermal process.

The addition of propylene glycol (Composition B) to enhance the solubility did not provide any advantages in the physical stability of the product. Since propylene glycol can

be a harmful excipient for pediatric patients (15), we decided to discontinue the stability studies with Composition B after 6 months. The substitution of amlodipine maleate for amlodipine besylate did not improve the stability of the formulation.

Due to the unpleasant taste of amlodipine besylate, we had to increase the amount of sucrose syrup above the recommended range of 10-20% required for acceptable palatability (16). Although the use of cariogenic sweeteners, such as sucrose, should be restricted for chronic use in pediatric formulations, acceptance of the formulation will highly depend on how it tastes (14). The sucrose syrup concentration was therefore considered to be acceptable. The palatability of our formulation was later surveyed in a bioequivalence study in healthy adult volunteers, and on average rated between "not good, not bad" and "good" (17).

In conclusion, we have developed a well-validated amlodipine oral solution, suitable for the pediatric population and able to provide the required dosing flexibility. This formulation is preferable to manipulated commercial dosage forms and non-standardized extemporaneously compounded formulations. It is suitable for large-scale production as well as extemporaneous compounding, which is in many situations necessary for the pediatric population. Our formulation has already proven to be bioequivalent to 5 mg tablets (17) and is now being studied in the pediatric population in order to construct a population pharmacokinetic model.

ACKNOWLEDGEMENTS

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5

Formulating a poorly water soluble drug into an oral solution suitable for paediatric patients; lorazepam as a model drug

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ABSTRACT

Introduction

Many drugs are unavailable in suitable oral paediatric dosage forms, and pharmacists often have to compound drugs to provide paediatric patients with an acceptable formulation in the right dose. Liquid formulations offer the advantage of dosing flexibility and ease of administration to young patients, but drug substances often show poor aqueous solubility. The objective of this work was to study different solvents and matrices to design a liquid formulation for poorly water soluble drugs, using lorazepam as model drug.

Methods

Three different formulation strategies were explored to improve the solubility. Firstly, water-soluble organic solvents were used to improve the aqueous solubility directly, secondly, ionic surfactants were used to solubilise the model drug, and thirdly, complexation of lorazepam with cyclodextrin was studied. Specific attention was paid to excipients, adequate taste correction and palatability. For the final formulation, physical and chemical stability and microbiological quality were assessed for 12 months.

Results

An organic solvent based formulation, containing a mixture of polyethylene glycol and glycerol 85%, with a minimum amount of propylene glycol, proved to be physically and chemically stable. Development of the non-ionic surfactants formulation was discontinued due to taste problems. The cyclodextrin formulations were physically stable, but lorazepam content declined to 90% within five months. The final formulation contained in volume concentration (%v/v) 87% glycerol, 10% polyethylene glycol 400 and 3% propylene glycol. Orange essence was the preferred taste corrector. The formulation remained stable for 12 months at 4°C, with lorazepam content remaining > 95%. Related substances increased during the study period but remained below 2%. In-use stability was proven up to 4 weeks.

Conclusion

An organic solvent based oral formulation was shown to be superior to a non-ionic surfactant based formulation or a cyclodextrin formulation. These results may help to formulate paediatric formulations of other poorly water soluble drugs, to aid pharmacy compounding.

INTRODUCTION

Many drugs are unavailable in suitable oral paediatric dosage forms (1), therefore, pharmacists often have to compound drugs to provide paediatric patients with an acceptable formulation in the right dose. In the reflection paper released by the paediatric working party of the European Medicines Agency (EMA) on formulations of choice for the paediatric population, solutions/drops and effervescent dosage forms are considered to have the highest applicability in a population of young patients (2). Capsules can be compounded extemporaneously in the dosage needed, but they need to be dissolved before administration and are difficult to administer through feeding tubes. Another disadvantage of extemporaneously compounded capsules is the difficulty in obtaining adequate content uniformity at low dosages.

Liquid formulations have the advantage of dosing flexibility and a reduced risk of choking. They can also be applied in other populations, such as geriatric patients with swallowing difficulties, or in a palliative setting. Possible disadvantages of liquid formulations are issues with stability and palatability, parameters that need to be considered in the design. As an alternative for liquid formulations, the development of mini-tablets has been given a lot of attention in the past years (3). They provide dosing flexibility and ease of administration, and generally solid formulations are more stable than liquid formulations. However, for most compounding pharmacies, tableting is not an available technique. Liquid formulations are therefore still commonly applied by pharmacist that need to compound for paediatric patients, both on individual and batch scale.

Drug substances sometimes show poor aqueous solubility. The use of solubilizing excipients can improve this, but especially in the paediatric population, the use of excipients needs to be considered carefully, with respect to safety and palatability. The objective of this study was to explore different formulation strategies for a poorly water soluble drug substance, lorazepam was chosen as a model drug.

Lorazepam (7-chloro-5-(2-chlorophenyl)-3-hydroxy-2,3-dihydro-1H-1,4-benzodiazepin-2-one) is a benzodiazepine indicated for the treatment of generalized anxiety disorder and pre-surgical anxiety in patients from the age of twelve years (4). Off-label, it is applied in a wide range of indications and patient categories, because of its sedative and anticonvulsive activity and absence of active metabolites. Within paediatrics, it is administered to children from the age of one month for acute anxiety, sedation, chemotherapy induced- or associated nausea, status epilepticus or for weaning purposes (5).

Currently, no liquid dosage form of lorazepam is available in the EU. An extemporaneous suspension of 1 mg/mL, prepared from 2 mg tablets, distilled water, Ora-Plus® and Ora-Sweet®, has been proven to be chemically stable for up to three months when stored at 4°C (6). However, a subsequent study using this suspension proved that dosage measurement by paediatric intensive care nurses led to significant deviations from the intended dose (7). These inaccurate dosage measurements are less likely to occur in the case of an oral solution, but the physical and chemical characteristics of lorazepam make this a challenge.

There are different strategies to formulate a poorly water soluble drug substance into an oral solution. pH Adjustment can be used to ionize a compound, which generally will result in increased aqueous solubility. In the case of lorazepam (aqueous solubility 0.08 mg/ml) (8), with pKas of 1.3 and 11.5 (9), pH adjustment is not a feasible method to increase the solubility. It is also sensitive to hydrolysis in both acidic and basic environments (10)

and shows temperature-dependent degradation (11). Organic solvents can be used as an alternative to water, but specific attention has to be paid to safety in paediatric patients. A distinction can be made between water-soluble and water-insoluble organic solvents. Water-soluble co-solvents, like ethanol (lorazepam solubility 14 mg/ml) and propylene glycol (lorazepam solubility 16 mg/ml) (8), create a mixed aqueous/organic solution. These excipients are readily available and easy to process, but they can convey a risk of toxicity to children (2). A combination of water-insoluble organic solvents, such as medium-chain and long-chain triglycerides and oleic acid, can be used to disperse lipophilic drugs. Alternatively, a poor water-soluble drug can be solubilized using surfactants, like polysorbate 20 and 80 (Tween) or polyoxyl hydrogenated castor oil (Cremophor), to obtain micelles in an aqueous environment. Similarly, surfactants can be used to obtain a microemulsion, when combined with a polar solvent, an oil, and a cosurfactant. Lastly, complexation of poorly soluble drugs with cyclodextrins has been a strategy to increase the aqueous solubility and bioavailability of compounds, while at the same time masking the taste (12), an important aspect in the design of paediatric formulations.

The objective of this study was to explore different formulation strategies to process a poorly soluble drug substance into a clear oral solution, using lorazepam as a model drug. The formulation needed to be suitable for paediatric patients from the age of one month, and have adequate stability to allow for individual and batch production within the pharmacy.

MATERIAL AND METHODS

Materials

Lorazepam drug substance was bought from Fagron BV (Capelle a/d IJssel, The Netherlands) and Duchefa Farma BV (Haarlem, The Netherlands). Lorazepam related compound B and hydroxypropyl-β-cyclodextrin (HP-β-CD, substitution degree 0.6) were bought from Sigma-Aldrich Chemie BV (Zwijndrecht, The Netherlands). Lorazepam related compounds C and D were bought from USP Switzerland (Basel, Switzerland). Colour Reference Solutions Y were bought from Merck Millipore (Amsterdam, The Netherlands). Lorazepam drug substance and all other excipients were European Pharmacopoeia grade.

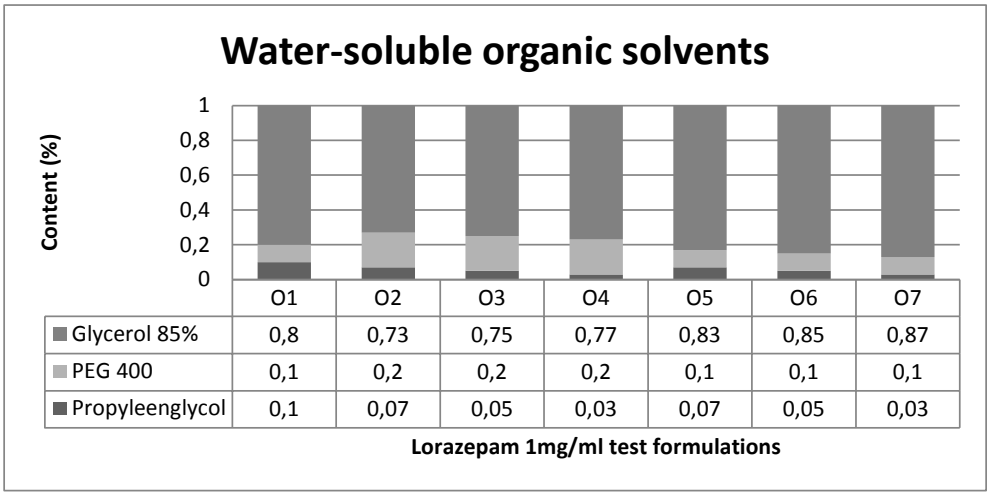
Formulation development

The dosage strength was chosen based on the target population of children from the age of one month to 18 years old, receiving a maximum dose of 0.6 mg/kg/day (5). To limit the volume needed and excipients administered, we aimed for a strength of 1 mg/ml. Three different formulation strategies were explored to improve the solubility. Firstly, water-soluble organic solvents were used to improve the aqueous solubility directly, secondly, non-ionic surfactants were used to solubilise the model drug, and thirdly, complexation of lorazepam with cyclodextrin was studied. Parameters that were studied were; physical stability (by visual inspection), chemical stability, using the analytical assay described in section 2.5, and palatability (see 2.3). Physical instability was defined as the presence of visible precipitation. The visual inspection of the samples was performed according to Ph. Eur. 2.2.1., with use of commercial reference solutions. The physical and chemical stability were initially studied for 5 months.

Organic solvents

For the organic solvents-based formulation, we experimented with different ratios of

propylene glycol (PG), poly ethylene glycol 400 (PEG400) and glycerol *85%*. Efforts were directed towards a glycerol/PEG400 based mixture containing minimal amounts of propylene glycol (Figure 5.1).



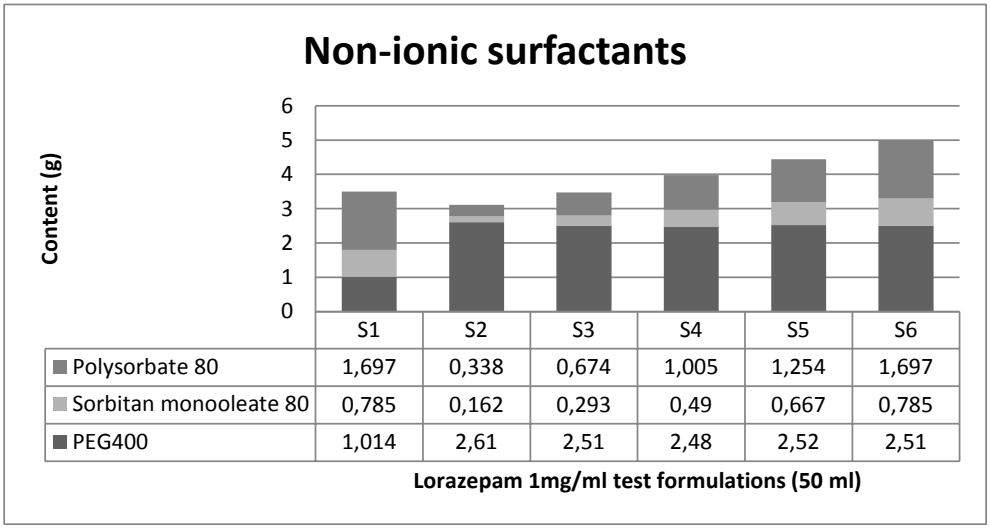
	O1	O2	O3	O4	O5	O6	O7
Glycerol 85%	0,8	0,73	0,75	0,77	0,83	0,85	0,87
PEG 400	0,1	0,2	0,2	0,2	0,1	0,1	0,1
Propyleenglycol	0,1	0,07	0,05	0,03	0,07	0,05	0,03

Lorazepam 1mg/ml test formulations

Figure 5.1 Lorazepam 1 mg/ml test formulations containing water-soluble organic solvents.

Non-ionic surfactants

The second strategy that was explored was the use of non-ionic surfactants to create a micellar solution. Polysorbate 80 and sorbitan monooleate were mixed in a ratio to obtain a hydrophilic/lipophilic balance (HLB) of 11.5. The total surfactant content in the test formulations ranged from 1-5%. PEG400 was used to dissolve lorazepam, after which the micellar solution was slowly added to the PEG400. The volume per test formulation was 50 mL, the composition of the excipients is displayed in Figure 5.2.



	S1	S2	S3	S4	S5	S6
Polysorbate 80	1,697	0,338	0,674	1,005	1,254	1,697
Sorbitan monooleate 80	0,785	0,162	0,293	0,49	0,667	0,785
PEG400	1,014	2,61	2,51	2,48	2,52	2,51

Lorazepam 1mg/ml test formulations (50 ml)

Figure 5.2 Lorazepam 1 mg/ml test formulations containing non-ionic surfactants.

Cyclodextrin

For the cyclodextrin formulation, HP-β-CD was chosen as the complexing agent, because of its high water solubility, lower cost compared to other cyclodextrins, low toxicity (12), and based on previous work investigating different cyclodextrins for inclusion complexation of lorazepam (13). A phase solubility diagram was made to measure the solubility of lorazepam as a function of the HP-β-CD concentration. This revealed that a minimum of 54 mg/mL HP-β-CD was required to obtain a 1 mg/ml lorazepam solution after 4 hours of ultrasonification. However, a HP-β-CD solution of 60 mg/mL (formulation C1) proved not sufficient to maintain a stable product after one week, therefor the HP-β-CD concentration was increased to 100 mg/ml (formulation C2). Glycerol 85% was added as a preservative in an amount of 35% m/v.

Palatability

The palatability of the test formulations was assessed by three adults, experienced in taste assessment. Characteristics that were evaluated were smell, taste, aftertaste and mouthfeel, and they were independently and qualitatively described by the taste panel. Taste correction possibilities were assessed with formulation C2, O6 and O7, using lemon, banana, raspberry and orange essence. Raspberry and banana were chosen as they are regularly applied in paediatric formulations. Lemon and orange flavours are good taste maskers for bitter drug substances.

Long-term stability studies

After the preliminary formulation studies, a decision was made to continue the development with formulation O7 (Table *3*). To this end, two batches of 3000 ml each were compounded, to investigate the influence of temperature and packaging material on long term stability. The test formulations were prepared with active pharmaceutical ingredient (API) from two different suppliers (Fabbrica Italiana Sintetici S.p.A and Cambrex Profarmaco Milano S.r.l.). Samples were stored in climate cabinets at 4 °C (VTL650K, range 2-8 °C) and 25°C 60% relative humidity (Elbanon type LC 500, range 23-27 °C, 55-65% RH) in amber-coloured polyethylene terephthalate (PET) and glass containers. In each cabinet the temperature was registered hourly. Because of the known temperature dependent degradation of lorazepam, stability studies at 40°C were omitted. Samples were tested against the release or end-of-shelf life specifications, based on the United States Pharmacopeia (USP) monograph for lorazepam oral concentrate and the general Ph. Eur. monograph for microbiological quality of non-sterile pharmaceutical preparations, shown in Table 5.1. Samples stored at 25°C were analysed at 0, 1, 2, and 3 months. Samples stored at 4°C were also analysed at 6, 9 and 12 months.

Table 5.1 Release and end-of-shelf life specifications.

Test Item	Method	Reference	Acceptance criteria
Identification	According to assay	Ph. Eur. Lorazepam Monograph	Spectra should be identical to reference
Appearance	Visual Observation	Ph. Eur. 2.2.1	Clarity ≤ Susp. I
		Ph. Eur. 2.2.2	Coloration ≤ Y5
Assay	HPLC-UV	Modified Ph. Eur. method	Lorazepam 90-110%
			Related compound C ≤ 4%
			Sum of other related compounds ≤ 2%
Microbiological quality	Bioburden filtration	Ph. Eur. 2.6.1.	E. Coli Absent
			TAMC (CFU/mL) < 100
			TYMC (CFU/mL) < 10

CFU = Colony-forming unit; TAMC = Total aerobic microbial count; TYMC = Total combined yeasts/moulds count

Analytical assay

For the quantitative analysis of lorazepam and lorazepam related compounds (USP) B, C and D [2-amino-2,5'-dichlorobenzophenone, 6-chloro-4-(o-chlorophenyl)-2-quinazolinecarboxaldehyde and 6-chloro-4-(o-chlorophenyl)-2-quinazolinecarboxylic acid, respectively] a high performance liquid chromatography combined with UV (HPLC-UV) detection method was used. The components were separated using a Shimadzu LC20 system, on a C18 analytical column (Inertsil ODS-3.5 µm 150x4.6 mm) with a mixture of acetonitrile, methanol and ammonium acetate solution (100 mM, pH 6.0 ± 0.04 adjusted with 1 M acetic acid) in the ratio 1:1:1 (v/v/v) as mobile phase, at a flow rate of 1.0 mL/min. Column temperature was kept at 30 ± 0.1°C and UV detection for quantification was performed at 230 nm using a Shimadzu M20A diode array detector, while the wavelength range of 200-400 nm was continuously monitored for unidentified peaks. The injection volume was 20 µl. The method was validated for the quantification of lorazepam in the cyclodextrin and PG/PEG 400/glycerol sample matrices and in the presence of related compounds B, C and D, for the parameters shown in Table 5.2. The response factors of related compounds B, C and D were determined to allow for accurate quantification of these compounds on lorazepam calibration curves.

Table 5.2 Validation parameters of the developed HPLC-UV analytical assay.

Parameter	Test	n	Specification	Result
Accuracy (12.5 - 37.5 µg/mL)	Recovery (%)	12	98.0 - 102.0	100.0
	Coefficient of variation (%)	12	< 1.0	0.5
Linearity (0 - 1.25 µg/mL)	F-value (12;1 p=0.05)	14	< 4.747	1.508
	Correlation coefiicient	14	> 0.9950	0.9978
Linearity (12.5 - 37.5 µg/mL)	F-value (10;1 p=0.05)	12	< 4.965	2.050
	Correlation coefiicient	12	> 0.9950	0.9997
Limits	LLOQ (µg/mL)	26	-	0.055
	LOD (µg/mL)	26	-	0.018
Intra-assay precision (0.25 µg/mL)	Coefficient of variation (%)	6	< 1.0	0.2
Intra-assay precision (25 µg/mL)	Coefficient of variation (%)	6	< 1.0	0.1
Inter-assay precision (25 µg/mL)	Coefficient of variation (%)	6	< 2.0	1.4
Response factors	Related compound B	4	-	0.707
	Related compound C	4	-	1.085
	Related compound D	4	-	0.999
Specificity	Lorazepam (%)	2	> 99.5	99.7
	Related compound B (%)	2	> 99.5	99.9
	Related compound C (%)	2	> 99.5	99.8
	Related compound D (%)	2	> 99.5	99.6

LLOQ lower limit of quantification, LOD limit of detection

Calibration and sample analysis

Samples were diluted 40 times to 25 µg/mL with mobile phase and quantified on a calibration curve (20–30 µg/mL) of freshly prepared standard solutions of lorazepam RS in mobile phase using the validated HPLC method. All duplicate sample analyses were preceded by a system suitability test consisting of replicate (n=5) injections of an equal

mixture of lorazepam RS 25 µg/mL in mobile phase and lorazepam related compound D, 25 µg/mL RS in mobile phase. Specifications for the relative standard deviation in the lorazepam peak areas and the resolution between the lorazepam and lorazepam related compound D peaks were ≤0.5% and 3.8-4.6, respectively. If unavailable, lorazepam related compound D can be created in situ by diluting a lorazepam RS 1000 µg/mL solution in methanol 40 times with 1 M sodium hydroxide and exposing it to a temperature of 70°C for two hours, then neutralized by mixing with an equal volume of 1 M hydrochloric acid.

In-use stability

An in-use test was performed on the final formulation (O7) based on a four-times daily dosing schedule. The containers were stored at 4°C (range 2-8°C) and based on the application in our PICU, four-times daily removed from the climate chamber to be exposed to air, light and ambient temperature for 15 minutes at every dosing simulation. Samples of 0.25 mL were withdrawn. After 28 days the samples were analysed in accordance with the specifications in Table 5.1. Microbiological quality was tested in accordance with the bioburden filtration method of Ph. Eur. 2.6.1.

Manufacturing procedure

The manufacturing procedure was developed with the intention to be suitable for individual and batch compounding. The lorazepam drug substance was levigated in a mortar with the solvent mixture. The remaining solvent was added by geometric dilution. Orange essence was added and the solution was magnetically stirred for one hour to achieve complete solution of the lorazepam.

RESULTS

Formulation development

The organic solvents-based formulations O1-O7 all resulted in physically stable products for at least 5 months. In formulation O1-O4, the lorazepam content declined to around 80-90% after 5 months at 4°C. Formulations O5-O7 were also chemically stable, with lorazepam content remaining around 100% after five months at 4°C. For this reason, we chose formulation O7, with the lowest propylene glycol content, to take into further development (Table 5.3).

Table 5.3 Composition of the lorazepam formulation studied for long-term and in-use stability.

Lorazepam	100	mg
Poly Ethylene Glycol 400	10	g
Propylene Glycol	3	g
Orange Essence	100	mg
Glycerol 85%	ad 108,1	g (=100 ml)

The surfactant-based formulations gave variable results. Formulations S1-S3 precipitated within a few days (S1) to two months (S3). Formulations S4-S6 remained physically stable during the study period. The content of S4 declined towards the end-of-shelf life limit of 90% within 3 months at 4°C. S5 and S6 remained chemically stable, but development of

these formulations was discontinued due to the bad soapy taste of the liquid.

The cyclodextrin formulation C2 containing 100 mg/ml HP-β-CD remained physically stable during the 5 month study period. The lorazepam content declined to around 90% after 5 months at 4°C with formation of related substance C up to 2,9%.

Palatability

The taste assessment results within the panel were consistent. Both cyclodextrin formulations had a neutral scent, slightly sweet taste, and a faint bitter taste caused by the lorazepam. There was no obvious aftertaste, but a prickly sensation on the tongue was sometimes observed. The lemon essence was the preferred taste corrector for formulation C2. Formulations S4 and S4 both had an overpowering soapy smell and taste, which was the reason for discontinuing the development of the surfactant-based formulations. All organic solvent-based formulations had a neutral scent, a sweet taste and a bitter aftertaste. Formulations with 20% PEG400 had a stronger bitter taste than formulations with 10% PEG400. Orange essence was the preferred taste corrector for formulation O6 and O7.

Long-term stability

The long-term chemical stability studies of formulation O7 showed that lorazepam content declined over time as displayed in Figure 5.3. A gradual increase in related compounds, mainly related compound C, was seen in all samples, but was notably higher at 25°C. Therefore, stability studies at 25°C were stopped after 3 months. At 12 months, related compound B was first measured in the 4°C samples and also an unknown impurity was found. Related compound C remained below 2.0%. The packaging material did not influence the chemical degradation of lorazepam. No changes in colour and clarity were observed in any of the samples.

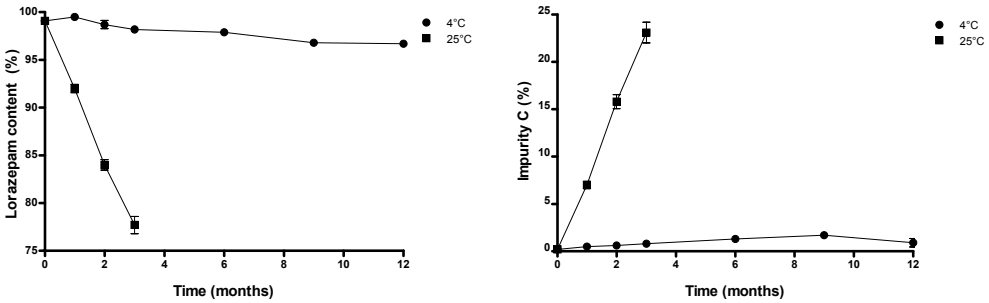


Figure 5.3 Average lorazepam content (left graph) with SD (n=4) and related compound C content (right graph) with SD (n=4) of formulation O7 at 4 and 25 °C .

In-use stability

The samples of formulation O7 remained stable during the in-use study, no visual changes were observed. The content of lorazepam did not decrease during the in-use study. Related substance C reached a maximum of 0.5% and the remaining related substances

were all below the quantification limit. The total aerobic microbial count and total yeast and mould counts were <1 colony forming unit per sample (the total remaining liquid per vial) at day 28 of the in-use study in all samples.

DISCUSSION

In this study, we explored different formulation strategies to compound a poorly water-soluble drug into a clear oral liquid formulation, using lorazepam as a model drug. With the intended application in paediatric patients, specific attention was paid to child-friendly excipients and adequate palatability. We developed an oral solution of lorazepam at a concentration of 1 mg/ml with adequate physical and chemical stability, and a shelf-life of at least 12 months. This clear solution can be expected to provide good dosing accuracy.

In our final, organic solvent based formulation, a small volume (3% m/v) propylene glycol was still needed to ensure adequate stability. Recently the European Medicines Agency has published a new assessment report concerning the safety of propylene glycol in paediatric formulations (14). In this report, new safety limits were set, expressed in terms of maximum daily doses that are considered to be safe whatever the duration and the route of administration. For neonates up to 28 days, this limit is set at 1 mg/kg, for children 1 month to 4 years old it is set at 50 mg/kg, and for children aged five years and up it is set at 500 mg/kg. Even in the rare occasion that the maximum dose of 0.6 mg/kg/day is required, the intake limits for patients above 28 days old will not be reached with our formulation. If administration to neonates is required, the propylene glycol limit of 1 mg/kg/day may be exceeded, and therefore its use is not recommended for neonates.

In the last decades, an increasing amount of research has been performed into cyclodextrins as a pharmaceutical excipient. The best known example of cyclodextrin in a commercial formulation, is itraconazole (Trisporal®) 10 mg/ml oral solution, containing 40% HP-β-CD and 2,5% propylene glycol, which is used off-label in children. HP-β-CD seems to be a promising option for a lorazepam solution. However, our results showed a restricted stability of maximum of 5 months, most likely due to hydrolysis of lorazepam. The compounding method, needing 4 hours of ultrasonification, proved impractical for individual preparations. The high amount of HP-β-CD required in this composition also makes it expensive. A possible solution that is currently being studied is the spray-drying of lorazepam-cyclodextrin 1:1 complexes, to provide a dry, and thus stable, semi-finished product, which can be compounded by pharmacist for individual patients.

Besides the technical challenges, there are also uncertainties around the safety of cyclodextrins in children below the age of 2 years. The oral bioavailability of HP-β-CD very low, and high doses could cause reversible diarrhoea. For children below the age of 2 years, the currently suggested permitted daily exposure of HP-β-CD is set at 16 mg/kg/day for oral ingestion (12). This is set at one tenth of the adult value, as there are insufficient data in this age group. It corresponds with a maximum allowable lorazepam intake of 0.16 mg/kg/day, which may be surpassed in clinical practice. In summary, a cyclodextrin formulation is a feasible option, but would require considerable additional research.

Our efforts to create a micellar solution of lorazepam resulted in a physically and chemically stable product, and the high amounts of surfactants required to obtain a stable solution would not exceed the Acceptable Daily Intake (ADI) limits for food additives set by the WHO (15, 16). However, the taste of the formulation made it unacceptable for use in children. The development of this formulation was therefore discontinued.

With regard to the palatability assessment by healthy volunteers, it is known that children experience different taste sensations than adults (17). In this stage of development we considered a first screening by an adult tasting panel acceptable. A palatability assessment is included in the clinical trial that is currently performed with our formulation in paediatric ICU patients.

In conclusion, we have studied different options for an oral solution of a poorly water soluble drug, using lorazepam a model drug. The organic solvent based formulation showed adequate stability, taste and dosing flexibility, rendering it suitable for the paediatric population above the age of one month. Our final, organic solvent-based formulation is currently used in a paediatric clinical trial to study the oral pharmacokinetics of lorazepam in PICU patients from the age of 1 month to 12 years old. This formulation is preferable to manipulation of commercial dosage forms and non-standardized extemporaneously compounded formulations, and may serve as an example for the development of comparable drug substances into oral liquid formulations.

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6

Biopharmaceutical tools in support of paediatric pharmacotherapy: an exploration using nifedipine and lorazepam

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ABSTRACT

AIMS

This study explores the impact of paediatric patient related factors on drug performance, with the use of biopharmaceutical tools; drug solubility in paediatric biorelevant media and biorelevant dissolution.

METHODS

Solubility studies addressed the influence of age and prandial state. Biorelevant dissolution experiments were designed to reflect clinical practice in a paediatric hospital, with respect to dosage forms, feeding regimens, and methods of administration. The compounds studied were nifedipine and lorazepam, both poorly soluble drugs and unionisable in the gastro-intestinal pH-range. Drug solubility and dissolution experiments were conducted in both adult and age-specific (neonate and infant) biorelevant media. Dissolution studies were performed with the mini-paddle apparatus and the flow-through cell apparatus.

RESULTS

A significant effect of food on nifedipine's solubility was observed, revealing the clinical importance since very young patients are almost continuously in a fed state. Dissolution of nifedipine formulations was not affected by age-related changes of the fasted state gastro-intestinal fluids, and by disintegration of the formulation before administration. A significant difference in nifedipine's dissolution rate from commercial tablets and compounded capsules was observed. A food effect on dissolution of lorazepam formulations was observed, but it was deemed less likely to be clinically relevant. Dissolution profiles of lorazepam tablets vs. lorazepam oral liquid were similar.

CONCLUSION

The *in vitro* results obtained from these studies reveal the potential of biorelevant solubility and dissolution studies reflecting clinical practice to predict drug performance in paediatric patients.

INTRODUCTION

During the last decades, the purpose of dissolution testing of drugs has expanded from pure quality control to prediction of *in vivo* drug performance and identifying potential bioavailability problems of pharmaceutical formulations. With development of biorelevant dissolution media (reflecting the main properties of gastrointestinal fluids), simulation of gastro-intestinal residence times, and simulation of gastro-intestinal hydrodynamics, numerous successful *in vitro in vivo* correlations (IVIVCs) have been established for oral immediate and modified release formulations in adults, using simple compendial dissolution apparatus (1, 2). Furthermore, *in vitro* dissolution data are used as input in physiologically based pharmacokinetic (PBPK) prediction models in which different factors influencing drug absorption are integrated. Both biorelevant dissolution and PBPK modelling are regularly applied in drug development by the pharmaceutical industry, i.e. to select and optimize formulations, or to predict solubilisation and precipitation in the human gastro-intestinal tract under various conditions (3). Ideally, these *in vitro* predictive methods, combined with *in silico* models, will more and more replace *in vivo* experiments and clinical trials.

In recent years, advances towards the availability of suitable paediatric biorelevant dissolution tests have been made with the development of paediatric biorelevant media (4). These media were based on the available literature on the composition of paediatric luminal fluids and the established adult media (5). Experimental dissolution parameters that mimic paediatric conditions have been proposed (6, 7). The search for alternatives to clinical trials is essential, especially in paediatrics, given the ethical and methodological difficulties involved in performing trials in paediatric patients (8).

With the adoption of the Paediatric Regulation, all applications for marketing authorisation for new paediatrics medicines have to include the results of studies as described in an agreed paediatric investigation plan (PIP). This PIP must include the development of an age-appropriate preparation for all relevant age groups, and additionally, alternative strategies for administration of the preparation (9). It is well known that drugs are not always administered as intended, to ease administration to children (10). Often, co-administration with food is recommended, but these strategies need to be evaluated, as vehicles for administration (e.g. food or drinks) can impact drug solubility and thus their bioavailability (11, 12). Predictive biopharmaceutical methods representing the *in vivo* drug dissolution in children would be of huge benefit for early formulation screening and assessing the influence of different administration strategies on drug performance, with the ultimate goal to reduce the amount of *in vivo* studies required and accelerating paediatric drug development.

Although the Paediatric Regulation has been very successful in stimulating the development of medicines for children, with 131 Paediatric Investigation Plans completed at the end of 2016, the development of age-appropriate formulations for off-patent medicines has not taken off (13). This means that in clinical practice, pharmacists still have to rely on extemporaneous compounding and unlicensed manufacturing or manipulation of adult dosage forms. Additionally, our knowledge on relevant pharmacokinetic parameters, including absorption, is often solely based on adult data. Apart from the obvious differences between children and adults regarding the physiological development of the gastro-intestinal tract, the effect of pharmacotherapy in paediatric patients can be further influenced by a range of patient-related factors, such as feeding regimens, the presence

of feeding tubes, immobility, and the selected drug formulation.

To help improve paediatric pharmacotherapy, this study aims to explore the application of biopharmaceutical methods to study the impact of patient related factors on drug performance in paediatric patients, using two biopharmaceutical tools; drug solubility in paediatric biorelevant media and biorelevant dissolution. To assess the age- and prandial state related changes in paediatric gastrointestinal solubility, solubility studies in both adult and paediatric biorelevant media were performed. Biorelevant dissolution experiments were designed to simulate the impact of formulation handling and dosage form manipulation. To reflect clinical practice in a paediatric hospital with respect to dosage forms, feeding regimens, and methods of administration, information from standard operating procedures of the Sophia Children's Hospital (Rotterdam, The Netherlands) was retrieved. Dissolution parameters were set based on what is currently known about physiological conditions in the GI tract of children.

The compounds that were chosen to be studied were nifedipine and lorazepam, as they are both regularly applied in unlicensed formulations and in very young patients.. Nifedipine is a BCS class II drug with poor aqueous solubility (around 5-9 µg/ml) over the range of pH 2 to pH10 (14) and a logP value of 2.20 (15). With pKa values of -0.9 and 13, it is not ionisable in the gastro-intestinal pH range. Therefore, under physiologically relevant conditions, nifedipine acts as a neutral molecule and its solubility is independent of the pH of the medium (16). Lorazepam is a compound with a slightly better aqueous solubility (80 µg/ml) compared to nifedipine, and a logP value of 2.39 (17). With pKa values of 1.3 and 11.5 (18), it is also not ionisable in the gastro-intestinal pH range. Using the above stated solubility value, a dose number (D_0) ≤ 1 is calculated for dose strengths up to 20 mg, and thus lorazepam is considered as a highly soluble compound within the BCS.

MATERIALS AND METHODS

Materials

Pepsin from porcine gastric mucosa (powder, ≥400 units/mg protein), nifedipine drug substance (≥98% HPLC grade), lorazepam reference standard (≥98% HPLC grade) and Whatman GF/D (pore size 2.7 µm, 25 mm diameter) and GF/F (pore size 0.7 µm, 25 mm diameter) filters were purchased from Sigma–Aldrich (Dorset, UK). UHMW polyethylene 10 micron full flow cannula filters were bought from Quality Lab Accessories LCC (Telford, USA). Egg-lecithin (Lipoid E PCS) was purchased from Lipoid GmbH (Ludwigshafen, Germany). Sodium taurocholate (NaTc) was purchased from Prodotti Chimici e Alimentari S.p.A (Basaluzzo, AL, Italy). Cronus 13 mm regenerated cellulose (RC) syringe filters 0.45 µm were purchased from LabHut Ltd (Maisemore, UK). Aptamil 1 (Nutricia, Trowbridge, UK), SMA Wysoy Soya Infant Formula (SMA Nutrition, Gatwick, UK) and Ultra Heat Treated Standardised Whole Milk 3.6% fat (Sainsbury's, London, UK) were purchased from a local supermarket. Water was of Milli-Q grade. All other reagents and chemicals were of analytical grade and were used as received, without further purification.

Instrumentation

Equipment utilized in the current study included a R114 Rotavapor (Buchi, Flawil, Switzerland), a SevenCompact pH/Ion S220 pH meter (Mettler-Toledo AG, Schwerzenbach, Switzerland), Heraeus Fresco 17 and Heraeus Biofuge Primo R centrifuges (Thermo

Scientific, Hanau, Germany), an Agilent Technologies 708-DS (USP II) apparatus configured with Agilent TruAlign 200 ml vessels and Agilent electropolished stainless steel mini-paddles (Santa Clara, CA), a Sotax CE7 smart flow-through cell (USP IV) apparatus connected to a CP 7 Piston Pump (Sotax, Switzerland). The Agilent 1100 HPLC system consisted of a G1311A Quaternary Pump, G1315A DAD detector, G1316A Column Compartment, G1322A Degasser, G1329A Autosampler, G1330A Autosampler Thermostat and ChemStation® software (Agilent Technologies, Santa Clara, US).

Drug formulations

Drug formulations were selected from the formulary of the Sopia Children's Hospital. Commercial nifedipine retard 10 mg tablets (Centrafarm, Etten-Leur, the Netherlands), unlicensed GMP-grade nifedipine 1 and 5 mg capsules (Apotheek A15, Gorinchem, the Netherlands), commercial lorazepam 1 mg tablets (Mylan, Bunschoten, The Netherlands) and unlicensed GMP-grade lorazepam oral solution 1 mg/ml (Apotheek A15, Gorinchem, the Netherlands) were used. Nifedipine capsules were compounded from pure API into hard gelatine capsules using lactose as single excipient. Lorazepam oral solution 1 mg/ml contains glycerol *85*%v/v (87%v/v), polyethylene glycol (PEG) 400 (10%v/v) and propylene glycol (3%v/v) (19). All formulations used in this study are included in the formulary of the Sophia Children's Hospital, Rotterdam, The Netherlands.

Media used for solubility and dissolution studies

Simulated Gastric Fluid without pepsin (SGF *sp*) pH 1.2 and Simulated Intestinal Fluid without pancreatin (SIF *sp*) pH 6.8 were used in dissolution studies (20). Freshly prepared adult and age-specific (neonate and infant) biorelevant media were used in solubility and dissolution studies (Table 6.1) (4, 5, 20, 21).

Solubility studies

Nifedipine solubility studies were performed according to methods described by Maharaj et al. (4). In summary, for aqueous based media, an excess amount of nifedipine was added to 2 mL of medium, dwelled for 24 hours at a shaking water bath at 37°C, filtered through an 0.45 µm RC filter and diluted with fresh medium prior to HPLC-analysis. For the milk-based media, a drug extraction step was required, which consisted of a centrifugation step, precipitation of proteins with methanol, a second centrifugation and filtration of the resulting supernatant through an 0.45 µm RC filter. All solubility experiments were conducted in triplicate.

In vitro dissolution studies

Experimental set-up

Dissolution experiments were performed with the mini-paddle apparatus and the flow-through cell apparatus. The mini-paddle apparatus is particularly suitable for working with reduced fluid volumes, to better mimic intraluminal fluid volumes in the GI tract of paediatric patients (22). The flow-through cell apparatus (USP IV apparatus) offers the advantage of easily changing the medium and flow rate during an experiment, and maintaining sink conditions when operated in the open mode (23).

Table 6.1 Adult and paediatric biorelevant media used in solubility and dissolution experiments (4, 5, 20, 21).

	Fasted-state Simulated Gastric Fluid			Fed-state Simulated Gastric Fluid		
	Adult (FaSSGF)	Neonate (Pn-FaSSGF)	Infant (Pi-FaSSGF)	Adult (FeSSGF)	Neonate - Cow Formula (Pnc-FeSSGF)	Neonate - Soy Formula (Pns-FeSSGF)
Sodium Chloride (mM)	34.2	34.2	34.2	237.02	100.35	94.79
Sodium Taurocholate (uM)	80	20	60			
Lecithin (uM)	20	5	15			
Pepsin (mg/mL)	0.1	0.015	0.025			
Acetic Acid (mM)				17.12	7.25	7.25
Sodium Acetate (mM)				29.75	64.65	64.65
Milk:buffer				1:1	1:1	1:1
HCl/NaOH qs	pH 1.6	pH 1.6	pH 1.6	pH 5	pH 5.7	pH 5.7
pH	1.6	1.6	1.6	5	5.7	5.7
Osmolarity (mOsm/kg)	120.7 +/- 2.5	120.7 +/- 2.5	120.7 +/- 2.5	400	340	240
Buffering Capacity (mmol/L/ ΔpH)	-	-	-	25	15	15
	Fasted-state Simulated Intestinal Fluid			Fed-state Simulated Intestinal Fluid			
	Adult (FaSSIF-V2)	FaSSIF-50%	FaSSIF-150%	Adult (FeSSIF-V2)	Neonate - Breast Fed (Pnb-FeSSIF)	Neonate - Cow Formula (Pnc-FeSSIF)	Infant - Cow Formula (Pi-Fessif)
Sodium hydroxide (mM)	34.8	34.8	34.8	81.65	81.65	81.65	81.65
Sodium Taurocholate (mM)	3	1.5	4.5	10	2.5	2.5	7.5
Lecithin (mM)	0.2	0.1	0.3	2	0.5	0.5	1.5
Sodium Chloride (mM)	68.62	68.62	68.62	125.5	95	111.73	107.35
Maleic acid (mM)	19.12	19.12	19.12	55.02	55.02	55.02	55.02
Glyceryl monooleate (mM)				5	5	6.65	5
Sodium monooleate (mM)				0.8	0.8	1.06	0.8
HCl/NaOH qs	pH 6.5	pH 6.5	pH 6.5	pH 5.8	pH 5.8	pH 5.8	pH 5.8
Osmolarity (mOsm/kg)	180 +/- 10	180 +/- 10	180 +/- 10	300 +/- 10	330 +/- 10	330 +/- 10	390 +/- 10
Buffering Capacity (mmol/L/ ΔpH)	10	10	10	25	25	25	25

The mini-paddle apparatus was equipped with 200 ml vessels and matching paddles, using a smaller volume compared to adult biorelevant studies (1). As intestinal motor activity matures throughout early infancy (24), the agitation rate of the paddle was set relatively low at 50 rotations per minute (RPM). 2 ml samples were removed (with sample replacement) using a 5mL Fortuna Optima® syringe fitted with stainless tubing and a cannula filter to facilitate representative sampling.

The flow-through cell apparatus was equipped with large cells (22.6 mm diameter), with a 5 mm ruby bead at the bottom of the cell and small glass beads (1 mm diameter) filling the cone of the cell. Test formulations were placed on a tablet holder. On top of each cell, two filters were placed; a GF/D and a GF/F filter (Glass Microfibre Filters 24 mm, Whatman™). In all experiments, the open mode was used. Samples were collected in glass cylinders or Erlenmeyer flasks, which were weighed to determine the volume of the sample.

All experiments, both in the mini-paddle and the flow-through cell apparatus, were conducted at 37°C. Sample collection for nifedipine took place at 5, 15, 30, 40, 50, 60, 75, 90 and then every 30 minutes up to 270 minutes, after the start of the experiment. Sample collection for lorazepam took place at 5, 15, 30, 45, 60, 75, 90 and 120 minutes after the start of the experiment. Before HPLC-analysis, samples were filtered through a 0.45 µm RC filter, discarding the first 10 drops (adsorption of the drugs onto the filters was checked and confirmed to be negligible). Calibration curves were prepared in corresponding media for each experiment on the day of the experiment. All experiments were performed in triplicate and in the case of nifedipine under protection from light.

Screening the impact of patient related variables

A clinically relevant design of the in vitro experiments was followed by using as input: i. the enteral feeding protocol, ii. the protocol for administration of medicines through a feeding tube, and iii. the local drug formulary of the Sophia Childen's Hospital (Rotterdam, The Netherlands). The patient relevant parameters studied in the dissolution experiments were age, prandial state, method of administration, and formulation type.

Nifedipine dissolution studies

An overview of the dissolution experiments is given in Table 6.2. Firstly, pH changes in the fasted state, resulting from passage through the stomach and small intestine, were simulated in the mini-paddle apparatus using SGF*sp* and SIF*sp* (see section 2.4). The pH shift from the gastric to the intestinal conditions was achieved by the addition of an equal volume of double concentrated SIF*sp* (with additional NaOH), after a simulated gastric residence time of 45 minutes. Secondly, the effects of age-related differences in gastro-intestinal conditions on dissolution were simulated with the use of biorelevant neonatal (Pn-FaSSGF/FaSSIF) and infant (Pi-FaSSGF/FaSSIF) fasting media in the mini-paddle apparatus (see Table 6.2). To reflect the *in vivo* conditions in neonates, the gastric residence time was prolonged, and the gastric volume was decreased compared to infants (8). Thirdly, administration through a gastric feeding tube, where the capsule is dispersed in an oral syringe with 5 ml of warm water, was also simulated in fasted state neonatal media (Pn-FaSSGF/FaSSIF). Although no dosing advice is available for neonates, in clinical practice nifedipine is sometimes administered to patients below the age of one month, in dosages from 0.1 mg/kg (25). For experiments simulating neonatal or infant conditions, nifedipine unlicensed 1 mg capsules were used to reflect clinical practice.

Table 6.2 Parameters used for the dissolution experiments in the mini-paddle and the flow-through-cell apparatus (USP IV apparatus).

				Gastric Conditions				Intestinal conditions					
Apparatus	API	Formulation	Agitation (rpm)	Medium	pH	Volume (ml)	Time (min.)	Medium	pH	Volume (ml)	Time (min.)	Total Volume (ml)	Total Time (min.)
Mini-paddle	Nifedipine	Capsule 5 mg A15	50	SGF*sp*	1.2	100	45	SIF *sp*	6.8	100	225	200	270
		Capsule 1 mg A15	50	Pn-FaSSGF	1.6	50	45	FaSSIF	6.5	150	225	200	270
		Capsule 1 mg A15	50	Pi-FaSSGF	1.6	75	30	FaSSIF	6.5	125	240	200	270
		Capsule 1 mg A15, dissolved in syringe	50	Pn-FaSSGF	1.6	50	45	FaSSIF	6.5	150	225	200	270
	Lorazepam	Oral solution 1 ml (1 mg/ml) A15	75	SGF*sp*	1.2	100	45	SIF *sp*	6.8	100	75	200	120
		Tablet 1mg Mylan	75	SGF*sp*	1.2	100	45	SIF *sp*	6.8	100	75	200	120
				Medium	pH	Flow (ml/min)	Time (min.)	Medium	pH	Flow (ml/min)	Time (min.)	Total Volume (ml)	Total Time (min.)
Flow-through cell	Nifedipine	Capsule 1mg A15		Pi-FaSSGF	1.6	4	30	FaSSIF	6.5	3	240	840	270
		Capsule 2x5 mg A15		FaSSGF	1.6	5	30	FaSSIF	6.5	4	240	1110	270
		Tablets retard 10 mg Centrafarm		FaSSGF	1.6	5	30	FaSSIF	6.5	4	240	1110	270
	Lorazepam	Tablet 1mg Mylan		Pi-FaSSGF	1.6	4	30	FaSSIF	6.4	3	90	390	120
		Tablet 1mg Mylan		Pnc-FeSSGF	5.7	5	60	Pi-FeS-SIF	5.8	5	60	600	120
		Tablet 1mg Mylan						Pi-FeS-SIF	5.8	5	120	600	120

API = active pharmaceutical ingredient, rpm = rotations per minute

To compare the dissolution of the two nifedipine formulations that are regularly used in the Sophia Children's Hospital, Centrafarm nifedipine retard 10 mg tablets and unlicensed 5 mg capsules, an experiment was performed in the flow-through cell apparatus. To simulate administration to fasted children, adult biorelevant media were used (FaSSGF/FaSSIF), the gastric residence was set at 30 minutes and the flow rate was reduced from 5ml/min to 4 ml/min after the medium switch in order to reflect the in vivo gastric and intestinal conditions (in terms of residence time and volume). The dose of the 10 mg tablets was matched by using two 5 mg capsules per cell. Lastly, a dissolution experiment in fasted state neonatal media (Pn-FaSSGF/FaSSIF) was performed with the flow-through cell apparatus, in order to reveal the effect of different hydrodynamics compared to the mini-paddle apparatus.

Lorazepam dissolution studies

Details of the design of the dissolution studies are presented in Table 6.2. Dissolution of the lorazepam 1 mg/ml oral solution and the 1 mg Mylan tablet formulation in SGF*sp* and SIF*sp* was performed with the mini-paddle apparatus (with an increased rotational speed of 75 rpm to prevent coning of the tablet formulation).

Subsequently, three experiments were conducted with the flow-through cell apparatus. The effect of prandial state on dissolution was explored using lorazepam 1 mg tablets and infant fasted state and fed state simulated gastric and intestinal fluids. Pnc-FeSSGF was considered appropriate to simulate infant fed-state gastric fluid, as it contains milk formula that is given to infants up to the age of six months. Gastric residence time in the fed state was prolonged compared to the fasted state and the flow rates were set to reflect the prandial state and gastro-intestinal tract compartment. Administration directly into the duodenum through a transpyloric feeding tube was simulated using lorazepam 1 mg tablets and infant fed state intestinal fluid (Pi-FeSSIF).

Analytical quantification

For the quantitative analysis of nifedipine, high performance liquid chromatography combined with UV (HPLC-UV) detection was used. The method was adapted from the method previously reported by Vertzoni *et al. (26, 27)*. Nifedipine was separated on an analytical C_{18} column (Thermo Hypersil GOLD, 5 µm, 250 × 4.6 mm) with UV detection at 238 nm, a column temperature of 30°C, mobile phase of a 60:40 mixture (v/v) of methanol and water (Milli-Q), a flow rate of 1.0 ml/min, and injection volume of 50 µl. For the quantitative analysis of lorazepam, the HPLC-UV method as reported by Share *et al.* was used (28). Lorazepam was separated using a Zorbax SB-C18 analytical column (3.5 µm, 150 × 4.6 mm) with UV detection at 230 nm, a column temperature of 30°C, mobile phase of a 60:40 mixture (v/v) of methanol and water (Milli-Q), a flow rate of 0.75 ml/min, and injection volume of 20 µl. Quantification of nifedipine and lorazepam was made based on calibration curves constructed from stock solutions in the corresponding medium (range 0.5-12 µg/ml). For milk- and formula-based media, calibration curves were created in triplicate, and the same protein precipitation, centrifugation and filtration process was applied as described in section 2.5.

Statistical analysis

One-way analysis of variance (ANOVA) with a post-hoc Tukey's test was applied to identify statistically significant differences in solubility between adult and age-specific media,

using a significance level of $p \leq 0.05$. Statistical analysis was performed in GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, CA).

RESULTS AND DISCUSSION

Clinical parameters: feeding regimens at the paediatric hospital

Even though oral administration is the preferred route to feed paediatric patients, enteral feeding through a nasogastric tube is often indicated, due to an inability or unwillingness of eating or swallowing, anorexia, motility problems etc. This mode of administration has implications for the gastric-emptying rate, which increases with enteral feeding compared to oral feeding (29). When possible, breast milk is the preferred type of food for children for a minimum duration of 4-6 months from the day of birth. Otherwise, patients are fed with formula milk, adjusted to their energy and protein requirements and potential fluid restriction. Table 6.3 displays the standard neonate and infant formulas administered to enterally fed patients. For normal birth-weight neonates and infants, the feeding interval is gradually reduced from 8 times a day at birth to 4 times a day at 8 months old. In certain conditions, such as gastroparesis, hyperemesis or recurrent aspiration, gastric feeding is not suitable and transpyloric feeding directly into the duodenum is required. Because the duodenum has no reservoir capacity like the stomach, transpyloric feeding is always administered as a continuous drip.

Table 6.3 Neonate and infant nutrition.

				PICU non ventilated		PICU ventilated	
Weight (kg)	Age (months)	Standard	Caloric content per 100 ml	Standard + energy enriched	Caloric content per 100 ml	Standard + energy and protein enriched	Caloric content per 100 ml
2-3.5	0-1	Nenatal Start	78 kcal 2.5 g protein	Nenatal Start 18% w/v	87 kcal 2.7 g protein	Nenatal Start 18% w/v + 0.5% NPF	89 kcal 3.2 g eiwit
3.5-8	0-6	Nutrilon® 1	66 kcal 1.3 g protein	Nutrilon® 1 17% w/v	82 kcal 1.6 g protein	Infatrini®	100 kcal 2.6 g protein
8-9.5	7-9	Nutrilon® 2	68 kcal 1.4 g protein	Nutrilon® 2 17% w/v	79 kcal 1.6 g protein	Infatrini®	100 kcal 2.6 g protein
9.5-10.5	10-12	Nutrilon® 3	70 Kcal 1,5 g protein	Nutrilon® 3 17% w/v	79 kcal 1.7 g protein	Infatrini®	100 kcal 2.6 g protein

Nutrilon® = Aptamil® first milk, NPF= Nutrilon® Nenatal Protein Fortifier, PICU = paediatric intensive care unit

All products are manufactured by Danone (Paris, France)

Paediatric Gastrointestinal Solubility of nifedipine

Nifedipine is a substance with poor aqueous solubility (around 5-9 µg/ml) (14). As shown in Figure 6.1, nifedipine solubility in adult FaSSGF (3.81 µg/ml) was statistically different ($p \leq 0.05$) to solubility in Pi-FaSSGF (7.04 µg/ml), but not to Pn-FaSSGF (4.99 µg/ml). Although a relatively large difference between the solubility values for adult FaSSGF and Pi-FaSSGF was observed, both were very similar to the range reported for aqueous solubility. This implicates that the small amounts of bile salts and pepsin present in FaSSGF have a negligible effect on nifedipine solubility, and that age-related changes in fasted state gastric fluid are unlikely to significantly influence the absorption of nifedipine.

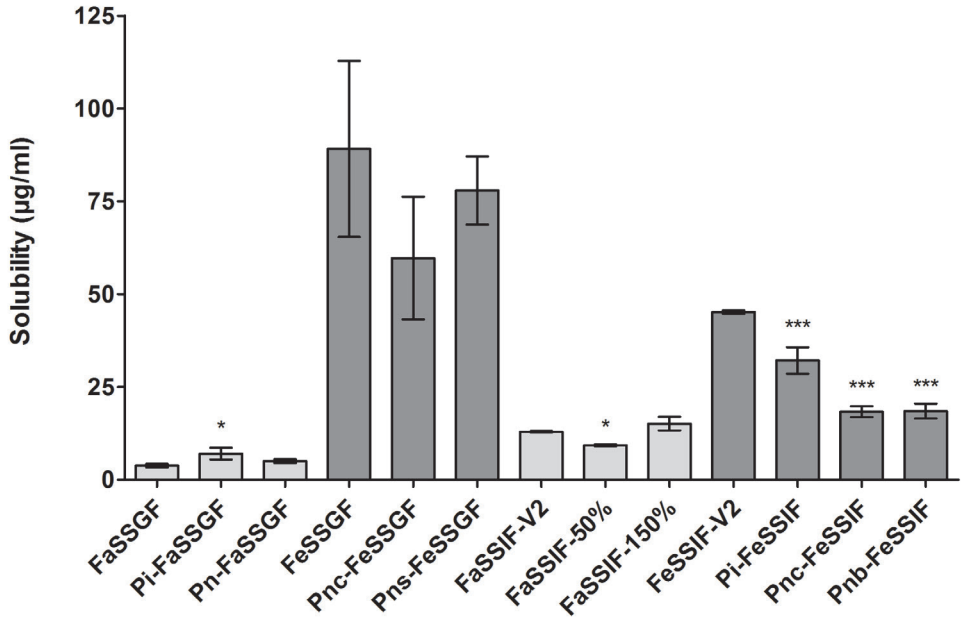

Figure 6.1 Nifedipine 24h solubility (mean±SD, n=3) in adult and paediatric biorelevant gastrointestinal media. Statistically significant solubility differences compared to the adult media are denoted with * ($p \leq 0.05$) or *** ($p \leq 0.001$). p=paediatric, i = infant, n = neonate, c = cow milk formula, s = soy milk formula, b = breast fed.

In FeSSGF, nifedipine solubility was markedly increased compared to FaSSGF, indicating a large effect of prandial state on nifedipine solubility in the gastric fluid. However, no statistically significant differences were observed between adult FeSSGF and paediatric FeSSGF, due to the high standard deviations. Even though the results were not statistically significant, the added meal component in FeSSGF is likely to influence the solubility of nifedipine. Additional solubility changes can be expected for energy and/or protein enriched nutrition (12).

Paediatric investigations examining luminal fluids within the fasted-state proximal intestine are thus far limited, therefore the different FaSSIF media were developed to explore the impact of variations in bile salt concentrations (4). The nifedipine solubility values that were found in FaSSIF-V2 (12.9 µg/ml), FaSSIF-50% (9.3 µg/ml) an FaSSIF-150%

(15.1 µg/ml) reflected these variations. A statistically significant difference ($p \leq 0.05$) was observed between FaSSIF-V2 and FaSSIF-50%, but not between FaSSIF-V2 and FaSSIF-150%. Compared to FaSSGF, solubility in FaSSIF was increased.

The biggest relative differences in solubility between adult and paediatric media were observed in FeSSIF. Nifedipine solubility in adult FeSSIF-V2 (45.2 µg/ml) was statistically different ($p \leq 0.001$) to Pi-FeSSIF (32.1 µg/ml), Pnc-FeSSIF (18.3 µg/ml) and Pnb-FeSSIF 18.5 µg/ml), reflecting the solubilizing effects of lipids and bile salts.

The increase in solubility in fed-state media has several implications for clinical practice. Both formulations that were studied are presented as slow release products, but are formulated as immediate release products. The slow dissolution rate of nifedipine itself is assumed to be the rate-limiting step in the onset of action. Multiple studies have shown that the bioavailability of nifedipine from film-coated and matrix tablets can significantly increase in the presence of food, and even dose-dumping can occur (30). In paediatric hypertension, nifedipine is administered to patients from the age of one month, who are effectively in an almost continuous fed prandial state. This means that a much more rapid release from the studied nifedipine formulations could be expected, possibly leading to a shortened Tmax, an increased Cmax and an increased bioavailability, compared to administration to fasted state patients. This may lead to typical dihydropyridine adverse effects like headache and flushing. More serious adverse events reported in paediatric patients possibly caused by nifedipine included change in neurological status, severe hypotension, and oxygen desaturation (31). Although the incidence of these adverse effects was low, the use of immediate release nifedipine is not recommended in the Netherlands (25).

Paediatric biorelevant dissolution studies

Nifedipine

The nifedipine dissolution results under paediatric biorelevant conditions in both the mini-paddle apparatus and the flow-through cell apparatus are presented in Figure 6.2.

Gastro-intestinal pH Figure 6.2 A shows the dissolution profile of a 5 mg unlicensed capsule in SGF *sp* and SIF *sp* in the mini-paddle apparatus. As nifedipine is unionisable in the gastro-intestinal physiological pH range, no apparent effect on nifedipine's dissolution from the pH switch was observed. A plateau was reached after around 180 minutes, with just over 20% of nifedipine dissolved. After the media switch, a rise in the amount dissolved was observed, resulting from an increased volume of dissolution medium. The large variability in the early phase of the experiment was caused by a variable capsule rupture time.

Age Figure 6.2 B shows the dissolution profiles of 1 mg unlicensed capsules in Pn-FaSSGF/FaSSIF-V2 and Pi-FaSSGF/FaSSIF-V2 in the mini-paddle apparatus. Between the dissolution profiles, a small effect of age was observed due to the difference in gastric emptying time/media switch, but an overall similar dissolution was seen at the end of the experiment. This was an expected result as dissolution conditions with regard to fluid composition only moderately differed in the gastric phase. The gastric release was again variable and low in both experiments, as a result of a variable capsule rupture time. Also, the bile salt content, an important constituent to nifedipine solubility, is much lower in (paediatric) FaSSGF compared to FaSSIF-V2 (4).

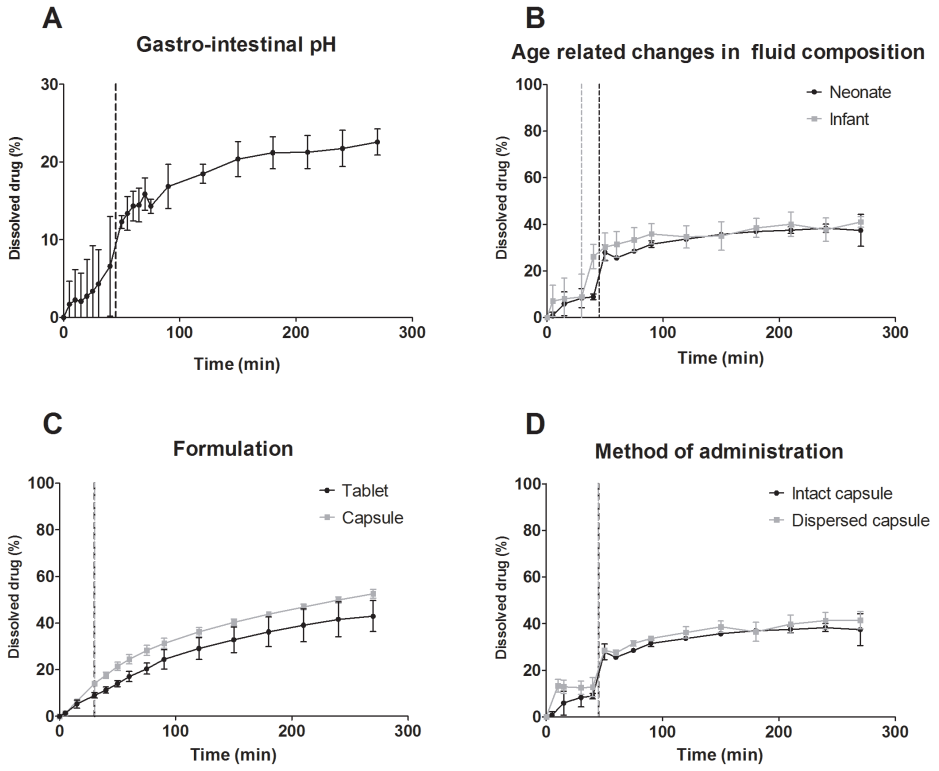

Figure 6.2 Dissolution profiles of nifedipine (mean±SD, n=3) under different conditions A: nifedipine 5 mg unlicensed capsules in SGF sp (45min)/SIF sp (225 min), mini-paddle apparatus (50 rpm), B: nifedipine 1 mg unlicensed capsules in Pn-FaSSGF (45 min)/ FaSSIF-V2 (225 min) (black circels) and Pi-FaSSGF (30 min)/FaSSIF-V2 (240 min) (grey squares), mini-paddle apparatus (50 rpm), C: nifedipine 10 mg Centrafarm slow release tablets (black circels) vs. 2x5mg unlicensed capsules (grey squares) in FaSSGF (30 min, 5 ml/min)/FaSSIF (240 min, 4 ml/min), flow-through cell apparatus, D: nifedipine 1 mg unlicensed capsules administered intact (black circels) vs. capsule content dispersed in water before administration (grey squares), in Pn-FaSSGF (45 min)/FaSSIF-V2 (225 min), mini-paddle apparatus (50 rpm). Dotted lines represent the time of medium change.

Formulation Nifedipine is commercially available in immediate release soft gelatine capsules, slow release tablets, and controlled release formulations. For paediatric patients of the Sophia Children's Hospital, nifedipine formulations are manufactured from raw material into low-dose capsules of 1 mg and 5 mg, and for higher dosages, generic controlled release and slow release tablets are prescribed (25). The dissolution profiles in FaSSGF/FaSSIF-V2 (simulating children) in the flow-through cell apparatus displayed in Figure 6.2 C show a different dissolution/release profile between commercial slow release 10 mg nifedipine tablets (Centrafarm) and the unlicensed nifedipine 5 mg capsules (Apotheek A15). The commercial tablets exhibited a slower dissolution and a much higher variability than the capsules. A likely explanation for these results is a difference in nifedipine particle size, which is an important physical factor influencing drug dissolution (32). In any case, the results suggest that these two formulations are not interchangeable and that bioavailability might not be the same.

Method of administration Administration of solid dosage forms to paediatric patients is often not possible, which means that the formulation has to be manipulated before administration. In the Sophia Children's Hospital, as per protocol, immediate release capsules and tablets are dispersed in an oral syringe with a small amount of lukewarm water (1-20 ml). Figure 6.2 D shows the dissolution profiles of nifedipine 1 mg unlicensed capsules administered intact vs. capsule content dispersed in water before administration in Pn-FaSSGF (45 min) and FaSSIF-V2 in the mini-paddle apparatus. Nifedipine's dissolution was slightly higher in the gastric phase in the case that the capsule content has been mixed with water before administration compared to the direct administration of the capsule, and profiles in the intestinal phase were similar (Figure 6.2 C). Since absorption mainly takes place from the small intestine and onwards, this suggests that a clinically relevant change from the different mode of administration of the capsule is unlikely (33).

Hydrodynamics Figure 6.3 shows the dissolution profiles of nifedipine 1 mg unlicensed capsules in Pi-FaSSGF/FaSSIF with the mini-paddle and the flow-through cell apparatus. Nifedipine's dissolution rate is clearly affected by the hydrodynamics. Dissolution of nifedipine 1 mg in the mini-paddle apparatus reaches a plateau of 40% after around two hours, due to the lack of sink conditions, whereas in the flow-through cell apparatus a continuous dissolution of nifedipine is observed with 70% dissolved at 270 min. For APIs with very low solubility, the flow-through cell apparatus would be preferred. Due to the continuous flow of fresh medium, sink conditions are achieved when the system operates in the open-loop configuration. In this way, the dissolution rate reflects the behaviour of the formulation and not the solubility of the substance, as in the closed systems (23).

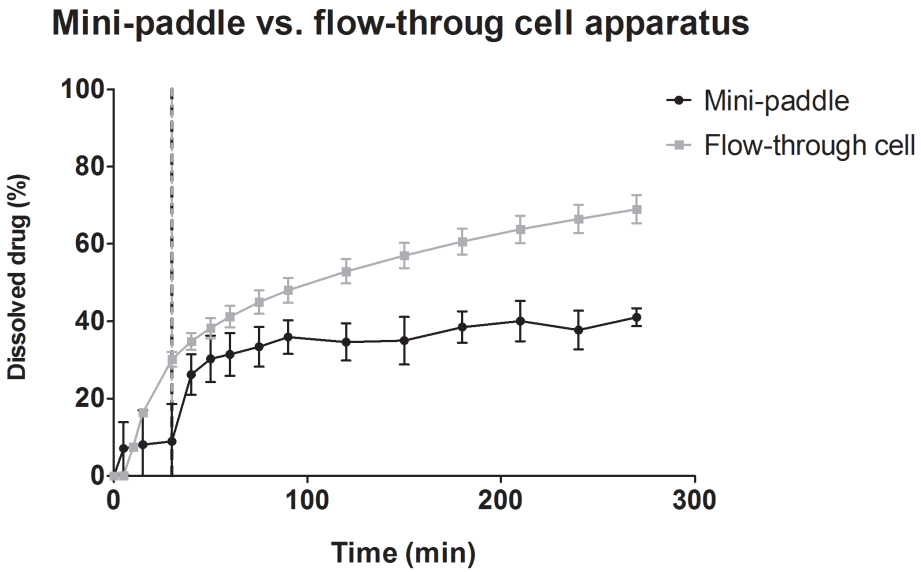

Figure 6.3 Dissolution profile of nifedipine 1 mg unlicensed capsules in Pi-FaSSGF (30 min)/ FaSSIF(240 min) in the mini-paddle apparatus (50 rpm) and the flow-through cell apparatus (4 ml/ min and 3 ml/min) (mean±SD, n=3). Dotted lines represent the time of medium change.

Lorazepam

The lorazepam dissolution results under paediatric biorelevant conditions in both the mini-paddle apparatus and the flow-through cell apparatus are presented in Figure 6.4.

Gastro-intestinal pH and formulation In paediatric patients, oral lorazepam is used (off-label) to gradually taper-off benzodiazepines that have been administered as continuous intravenous sedation at the paediatric intensive care unit (PICU). It is usually administered 4 times daily, in single dosages up to 0.5 mg (neonates), 1 mg (infants) and 3 mg (children). Until recently, the only available formulations were tablets, extemporaneous capsules, and Temesta 4 mg/ml solution for injection, which contains high amounts of unfavourable excipients. To allow for precise dosing and ease of administration, a 1 mg/ml oral solution was developed specifically for paediatric patients (19). Performance of the lorazepam oral solution 1 mg/ml (1 ml) and 1 mg Mylan tablet formulation was assessed in SGF *sp* and SIF *sp* with the mini-paddle apparatus (Figure 6.4 A). During the simulated gastric residence time (45 min), almost all lorazepam was dissolved from the tablet, reaching similar concentrations as compared to the lorazepam liquid after only 15 minutes. Within the clinical application of lorazepam for the prevention of withdrawal, large fluctuations in plasma concentrations are undesirable. The similar dissolution performance of the oral solution and tablet suggest that the administration of the liquid will not cause unexpectedly rapid absorption as compared to the tablet.

Prandial state Results from the flow-through cell apparatus dissolution studies depicted in Figure 6.4 B show the dissolution profiles of lorazepam 1 mg Mylan tablets in Pi-FaSSGF/FaSSIF-V2 and Pnc-FeSSGF/Pi-FeSSIF. A slower dissolution rate was observed in the fasted state, but overall a similar % dissolved was observed for the simulated fasted (81.3% +/-8.4%) and fed state (95.3% +/-3.0%) after 2 hours. The dissolution profiles confirm the *in vivo* observations, as a food effect has not been described for lorazepam in either adults or children.

Method of administration Sometimes a patient does not tolerate gastric feeding and transpyloric feeding directly into the duodenum is required. When necessary, medication is also administered through the duodenal feeding tube and solid dosage forms are crushed or dispersed. When direct administration of the 1mg Mylan lorazepam tablet to the duodenum was simulated in Pi-FeSSIF in the flow-through cell apparatus, the dissolution profile was similar to the one obtained in Pnc-FeSSGF/Pi-FeSSIF (Figure 6.4 C). These results suggest that administration through a duodenal feeding tube will not impact the in vivo dissolution. A change in t_{max} is still possible however, as lorazepam reaches the site of absorption, namely the upper intestine, more quickly than when it is administered orally or via a gastric feeding tube.

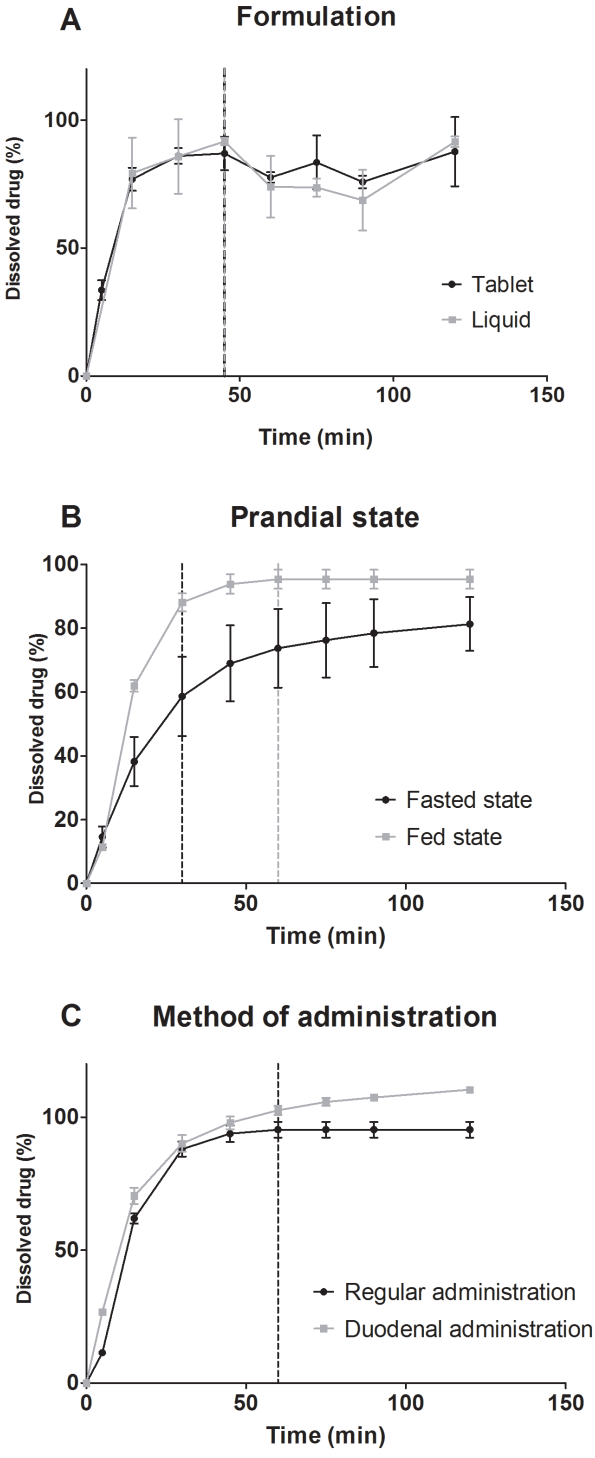

Figure 6.4 Dissolution profiles of lorazepam (mean±SD, n=3) under different conditions.

A: 1 mg Mylan tablet (black circles) vs. 1 ml unlicensed oral solution 1 mg/ml (grey squares), SGF sp (45 min)/ SIF sp (75 min), mini-paddle apparatus (75rpm).

B: 1 mg Mylan tablets, fasted-state Pi-FaSSGF (30 min, 4 ml/min)/FaSSIF-V2 (90 min, 3 ml/min) vs. fed-state Pn-FeSSGF (60 min, 5 ml/min)/Pi-FeSSIF (60 min, 5 ml/min), flow-through cell apparatus.

C: 1mg Mylan tablets, regular administration Pn-FeSSGF (60 min, 5 ml/min)/Pi-FeSSIF (60 min, 5 ml/min) vs. duodenal administration Pi-FeSSIF (120 min, 5 ml/min), flow-through cell apparatus. Dotted lines represent the time of medium change.

GENERAL DISCUSSION

This study yields information on the application of nifedipine and lorazepam in paediatric pharmacotherapy.

We observed a significant food effect on the solubility of nifedipine in paediatric media, which is in agreement with previously reported clinical data in adults (30). Our results suggest this influence of food on absorption of nifedipine is also applicable in paediatric patients, and needs to be taken into account when administering nifedipine to paediatric patients. Clinicians should be aware that they cannot rely on the slow onset of action associated with nifedipine when the studied formulations are administered to patients in the fed state. Furthermore, questions were raised about the bioequivalence of the nifedipine commercial tablets and unlicensed capsules. Reassuring results came from the experiment dispersing the nifedipine capsule in water before administration, a commonly applied administration technique, revealing no significant differences in dissolution and thus implying no altered bioavailability in comparison to administration of the intact capsule. As mentioned in section 3.2, markedly increased or accelerated absorption of nifedipine could lead to adverse effects such as severe hypotension, and must be avoided.

When administering lorazepam to PICU patients to prevent iatrogenic withdrawal syndrome, precise dosing is required (34). For this reason, and for ease of administration through a feeding tube, a liquid formulation would be the dosage form of choice. In our experiments, the dissolution of lorazepam from either tablets or the oral solution was less affected by the different experimental set-ups, namely the simulated prandial state and administration site. As there are no indications that lorazepam is a substrate to gastro-intestinal drug transporters, and absorption is almost complete in adults (35), it is also unlikely that excipients from the oral liquid will alter the lorazepam absorption compared to the tablets. The results from our study give reassurance about the interchangeability of liquid versus immediate release lorazepam tablets, and the negligible effects of prandial state and administration site on the *in vivo* performance of the lorazepam formulations.

Ideally, the results obtained from *in vitro* dissolution experiments would be integrated into more complex *in silico* prediction models, which are able to include other factors influencing absorption, like gastric emptying. This physiologically based pharmacokinetic (PBPK) modeling and simulation is already commonly used in formulation development/ bridging for adult medicines and provides a promising tool for paediatric *in vivo* drug performance prediction, provided we gain a better understanding of the developmental changes of the gastrointestinal tract in the paediatric population (3). Aside from the factors influencing *in vivo* dissolution, specific research is still required on the factors influencing permeability, mainly the ontogeny of metabolizing enzymes and drug transporters, to better predict oral drug absorption in this population (36). Ultimately, the developed biopharmaceutical tools could be validated using paediatric pharmacokinetic data, when available and possible to be shared by the pharmaceutical industry. The validated biopharmaceutical tools can then be used to study off-patent paediatric drugs that would otherwise be neglected.

There is still a knowledge gap concerning GI physiology in paediatric patients. With the development of the paediatric biorelevant media, extrapolations from adult values had to be made for some aspects when availability of paediatric data was limited (4). Future adaptations of the media compositions are therefore likely, when clinical investigations

yield more accurate paediatric values. Other aspects that would gain from future clinical information and would be further updated in the design of *in vivo* predictive dissolution tests for the paediatric population would relate to the fluid volumes available at the gastro-intestinal lumen, and the motility patterns and hydrodynamics. Slight changes to the experimental conditions presented in this study would not affect the overall results and conclusions for the studied formulations.

CONCLUSION

The *in vitro* results obtained from the experiments in this study, designed to reflect clinical practice in a paediatric hospital, show that biorelevant solubility and dissolution studies could assist in the prediction of drug performance in paediatric patients. The straightforward dissolution setups make it possible to address numerous different administration scenarios, which would not be feasible or ethical in pharmacokinetic studies in children.

FUNDING

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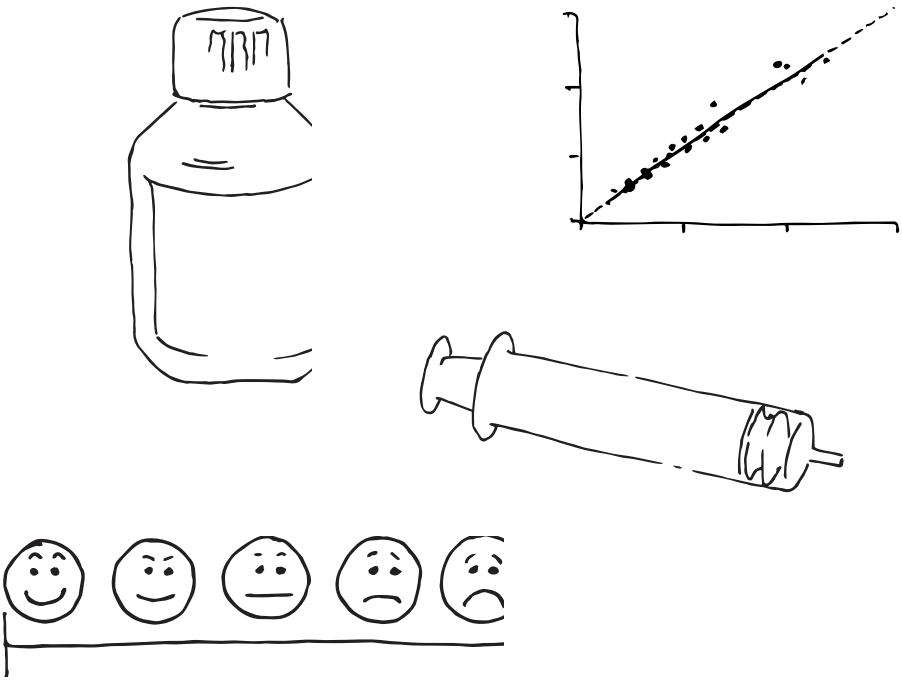
PART III

Clinical evaluation of the formulations



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Bioequivalence study of an extemporaneously prepared oral solution of amlodipine suitable for use in pediatric patients compared to commercial tablets

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ABSTRACT

Objective

Amlodipine, a long-acting dihydropyridine calcium channel blocker, is frequently prescribed to pediatric patients. To date, no suitable pediatric formulation has been available. In this study, an amlodipine oral solution was developed and tested for bioequivalence to tablets in healthy adult volunteers.

Methods

This study was designed as an open-label, single-dose, two-sequence, two-period, crossover trial to assess the bioequivalence of a newly developed amlodipine besylate oral solution 0,5 mg/mL compared to Norvasc® 5 mg tablets. Thirteen adult subjects (mean [standard deviation] age of 23.2 [3.6] years, weight 71.5 [7.7] kg) were included and blood samples were collected for 72 hours. Amlodipine plasma levels were determined using a validated UPLC-MS/MS assay. Non-compartmental pharmacokinetic parameters were compared between the formulations according to European Medicines Agency (EMA) bioequivalence guidelines.

Results

The 90% confidence intervals of the test/reference ratios of the geometric means for the primary pharmacokinetic parameters AUC_{0-72} (88.24 - 104.37%) and C_{max} (99.00 - 121.40%) were within the acceptance range of 80.00-125.00% for bioequivalence. Mean (SD) AUC_{0-72} was 102.7 (26.8) µg*h/L for the solution and 108.2 (30.6) µg*h/L for the tablet. Mean (SD) C_{max} of the solution was 3.11(1.06) µg/L with a median (IQR) T_{max} of 4.0 (2.6-7.5) hours. Mean (SD) C_{max} of the tablet was 2.91 (0.84) µg/L with a median (IQR) T_{max} of 6.0 (4.0-14.0) hours. Intrasubject coefficients of variation were 10.2% (AUC_{0-72}) and 12.4% (C_{max}).

Conclusions

The formulations are bioequivalent according to EMA guidelines. This warrants further study of our novel amlodipine oral solution in pediatric patients.

INTRODUCTION

Amlodipine is a long-acting dihydropyridine calcium channel blocker widely used in both adults and children. Currently, it is one of the antihypertensive agents recommended by the European Society of Hypertension for the management of hypertension in children and adolescents (1). Within the group of calcium channel blocking agents, amlodipine is considered first choice treatment for chronic hypertension in children, based upon its pharmacological characteristics and as the most extensively studied drug within this class (2). Its main advantage is its long half-life, enabling once or twice daily administration.

Amlodipine is officially licensed for treatment of children from the age of six, but also prescribed off-label to children from the age of 1 month in a dose of 0.06-0.3mg/kg per day (1). However, the commercial available formulations are limited to tablets of 5 or 10 mg. These tablets are not suitable for the youngest age group, in which lower dosages and higher dose flexibility are generally needed. A liquid formulation would therefore be a more appropriate dosage form for young patients. Some liquid formulations of amlodipine have been proposed, but have been composed as suspensions (3-5). For a drug that can be highly toxic when overdosed, especially in children (6), a suspension is not preferred. Suspensions can become inhomogeneous, leading to accidental administration of wrong dosages. For this reason we developed a solution of amlodipine, for oral pediatric use. We validated its stability and the formulation has shown to be stable for at least one year (7).

Amlodipine immediate release, solid dosage forms of ≤5 milligrams are classified in Biopharmaceutics Classification System class I by the WHO (8). This implies a high gastro-intestinal solubility and permeability of amlodipine. Given these characteristics, we expect our oral solution to be bioequivalent to amlodipine tablets according to European Medicines Agency (EMA) guidelines (9). However, with the test product being a solution, a shift in T_{max} might occur. To be able to safely apply the oral solution in the pediatric population, we chose to first elucidate the pharmacokinetic parameters of the oral liquid in adult volunteers.

In adults, amlodipine is slowly and completely absorbed after oral ingestion with peak plasma concentrations between 3 and 12 hours (10-12). As a result of its first-pass effect, tablets show high, but variable, bioavailability (50-90%), which is not influenced by food (10, 12, 13). Amlodipine is extensively metabolized in the liver, mainly by CYP3A4 (12). Initial metabolism involves the oxidation of the dihydropyridine ring to the pyridine analogue, complemented by side-chain oxidation and hydrolysis of one or both side-chain ester groups. Around 60% of amlodipine is excreted in the urine, with up to 5% in unchanged form (14). The half-life ranges between 30 and 50 hours, and seems to increase with age (10, 14).

In this study we investigate the bioequivalence of 10 mL amlodipine besylate 0.5 mg/mL in comparison with the innovator 5 mg Norvasc® (amlodipine besylate) tablet after a single oral dose in healthy volunteers, in a crossover design.

METHODS

Drug formulations

The quantitative composition of the amlodipine oral solution is shown in Table 7.1. Long-term stability studies have proven that the solution is stable for at least one year when stored at 4°C, with the contents of amlodipine besylate not dropping below 95% and related substances remaining below 0.4%. The reference treatment consisted of Norvasc® (Pfizer BV, Capelle a/d Ijssel, The Netherlands) 5 mg tablets, containing the excipients sodium starch glycollate (type A), calcium hydrogen phosphate, anhydrous, cellulose, microcrystalline and magnesium stearate.

Table 7.1 Composition of the test formulation amlodipine besylate oral liquid 0.5 mg/mL.

Composition of amlodipine oral liquid	Quantity
Amlodipine besylate	69.0 mg
Methyl paraben solution* 15% m/v FNA	304 mg
Sucrose syrup^	8.53 g
Purified water	Ad 100 mL

FNA Formulary of Dutch Pharmacists; * solution of methyl paraben in propylene glycol ^solution of 630 mg sucrose and 1 mg methyl parahydroxybenzoate in 1 g of water

Study population and Recruitment

From March to May 2013, we recruited healthy male and female volunteers in Rotterdam, The Netherlands. Subjects were eligible for inclusion if they met the following criteria: age between 18 and 55 years, Caucasian and body mass index from 19 to 25. All subjects were considered healthy on the basis of a physical examination and recording of medical history performed by a physician. Exclusion criteria for participation were: sitting blood pressure lower than 120 mmHg systolic and 80 mmHg diastolic in resting conditions, use of any other medication excluding contraceptives, smoking, pregnancy, history of alcohol or drug abuse, known hypersensitivity to dihydropyridine derivatives or any other contra-indication for amlodipine use.

The study was conducted in line with Good Clinical Practice and the Declaration of Helsinki and approved by the Medical Ethics Committee of the Erasmus Medical Centre and by the Dutch competent authority. Written informed consent was obtained from each subject. All subjects had the right to withdraw from the study at any time without any consequences. The trial was registered in the Dutch Trial Register.

Study design

The study was conducted in a single-center, randomized, open-label, two-sequence, two-period, crossover design at the Erasmus Medical Centre, Rotterdam, The Netherlands. We evaluated two single-dose treatments of amlodipine. Two sequences (test-reference and reference-test) were randomly allocated to subjects using the Trial Online Process (TOP) program of the HOVON data center, Rotterdam, The Netherlands. Administration of the study drug was not blinded, because of the difference of appearance of tablets and liquid

and because it was not deemed necessary for the purpose of the study.

A wash-out period of at least 14 days was maintained between test and reference treatment. An intravenous catheter was placed on the day of study drug administration to draw blood samples. Samples were taken at baseline and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 , 14, 24, 48 and 72 hours post dose. The samples at 24, 48 and 72 were drawn by venipuncture. The test product, 10 mL of amlodipine oral liquid 0,5 mg/mL, was dispensed in an oral syringe and ingested without additional fluid, to enable a taste assessment. The reference product (tablet) was ingested with tap water. The subjects underwent an overnight fast for at least eight hours. Water and tea were allowed before and during the study period. Standardized meals were provided at 1, 5 and 11 hours after administration of the study drug. Consumption of grapefruit juice and smoking was not allowed during the study.

For safety reasons, we monitored sitting blood pressure and heart rate of all subjects at baseline and 1, 3, 6, 8, 10, 12, 14, 24 48 and 72 hours post dose for both study drugs using an automated oscillometric device. Subjects were excluded if their systolic blood pressure dropped below 70 mmHg or if their diastolic blood pressure dropped below 40 mmHg. After administration of the test product, the subjects had to fill out a taste assessment form based on a five-point hedonic scale. We surveyed the subjects for adverse events during the study period and one week after.

Amlodipine analysis

Plasma concentrations of amlodipine were determined using a validated ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method, that was developed for the purpose of this clinical study.

Plasma samples were stored at -80°C until analysis. After thawing at room temperature, protein was precipitated by adding 600µl methanol, containing the internal standard (amlodipine-d4, Art Molecule, Poitiers, France), to 200µl plasma sample. After vortexing for ten seconds, samples were centrifuged at 15973 g for five minutes. After centrifugation 600µl supernatant was diluted with 150µl of methanol: water (50:50 v/v) and vortexed for 5 seconds. Samples were kept at room temperature until analysis. A 5-µl sample was injected onto the UPLC-MS/MS system (Thermo Scientific Dionex Ultimate 3000) consisting of an Ultimate 3000 RS quaternary UHPLC-pump, an Ultimate 3000 RS auto sampler and an Ultimate 3000 RS column compartment in combination with a Thermo Scientific TSQ vantage MS/MS for mass spectrometric detection. The auto sampler was kept at 15°C. Isocratic elution was achieved with a mixture of 65% of 2mM Ammoniumacetate + 0,1% formic acid in water (mobile phase A) and 35% of 2mM Ammoniumacetate + 0,1% formic acid in methanol (mobile phase B) at a flow rate of 0,4 ml/min. The column (Waters Acquity UPLC HSS T3, 2.1x100 mm, 1.8 µm) temperature was set at 40°C. The total runtime was 4 minutes. Compounds were detected using Electron Spray Ionization (ESI) in positive mode. The SRM transitions of Amlodipine and Amlodipine-d4 were 409 > 238 and 413 > 238 [M+H]+ respectively. Optimal MS settings were as follows: Spray Voltage 3000V, Capillary Temp 200°C, Vaporizer Temp. 400°C, collision gas pressure 1,5 mTorr, Sheat gas 50, Ion Sweep gas 5, Aux gas 20, Collision Energy 10V and S-lens RF amplitude 64. Data processing was performed with LcQuan 2.7 software (Thermo Scientific).

Satisfying results of intraday precision, interday precision and accuracy were conclusively

demonstrated during the period of method validation. Six replicates of three levels of quality control (QC) samples (0.493, 7.398 and 16.77 µg/L) were used to determine accuracy and intraday precision with the maximum coefficient of variation (%CV) set at 15 %. For interday precision three levels of QC samples (0.493, 7,398 and 16.77 µg/l) were analyzed in duplicate on six consecutive days. The maximum %CV was also set at 15%. The linear calibration curves were obtained in the concentration range of 0.2 to 20 µg/L using a weighing factor of 1/x. The correlation coefficient was 0.9965. The lower limit of quantification was 0.1 µg/L.

Pharmacokinetic and statistical evaluation

The intra-subject coefficient of variation (CV) for pharmacokinetic parameters was assumed to be 16% (4) and the geometric mean ratio (test/reference) of the pharmacokinetic parameters was assumed to be 1.05. We expected the minimum sample size of 12 evaluable subjects to be sufficient to reject the null hypothesis "lack of bioequivalence between test and reference treatment" with $\alpha = 0,05$ and a power of at least 80% (15). We performed the statistical analysis according to recommendations of the European Medicines Agency on the investigation of bioequivalence (9). The assessment of bioequivalence was based upon 90% confidence intervals for the ratio of the population geometric means of the test and reference formulation for the parameters AUC_{0-72} and C_{max}, equivalent to two one-sided tests with the null hypothesis of bioinequivalence at the 5% significance level. AUC_{0-72} was considered adequate for comparison of extent of exposure of the two immediate release formulations, as the absorption phase of amlodipine has been covered. We calculated the AUC_{0-72} using the linear trapezoidal rule. Extrapolation to infinity ($AUC_{0-\infty}$) was performed by dividing the last measurable serum concentration by the elimination rate constant (λz). ANOVA was carried out using the respective log-transformed data. The mean square error of ANOVA was used as a variance estimate to calculate the 90% CI. The predefined acceptance range was 80.00% to 125.00% for AUC_{0-72} and $C_{max.}$ The elimination half-life was determined from 0.693/λz. We estimated C_{max} values and T_{max} directly from the observed plasma concentration–time data. The software used for all calculations was Microsoft Excel 2010 (Microsoft Corporation, Seattle, Washington), IBM SPSS Statistics 21.0.0.1 (IBM Corporation, Armonk, New York) and WinNonlin 6.01 (Pharsight Corporation, Palo Alto, California).

RESULTS

A total of 13 subjects were enrolled in the study (4 male, 9 female, mean [SD] age of 23.2 [3.6] years; weight 71.5 [7.7] kg; height 177.5 [8.5] cm). One subject dropped out during study period 2, due to displacement of the intravenous catheter. After t=1.5 no evaluable blood samples were obtained and subsequently this subject was excluded from the pharmacokinetic analysis.

No serious adverse events occurred during the study period. The blood pressures remained above the threshold for exclusion in all subjects. One subject complained of headache in the week after the second treatment period, but this was not attributed to the study medication. Another subject suffered from the flu between treatment periods but was considered healthy at the start of the second study period.

Pharmacokinetics

The liquid test and the solid reference preparation showed similar pharmacokinetic properties. Figure 7.1 shows the mean amlodipine concentration versus time plots. Individual amlodipine concentration versus time plots are shown in Figure 7.2. No relevant pre-dose amlodipine concentrations were observed. An overview of the pharmacokinetic parameters of the two amlodipine dosage forms is given in table 7.2. Mean (SD) AUC_{0-72} was 102.7 (26.8) µg*h/L for the test product and 108.2 (30.6) µg*h/L for the reference product. Mean (SD) $AUC_{0-\infty}$ was extrapolated to 141.3 (50.3) µg*h/L for the test product and 147.4 (49.6) µg*h/L for the reference product. Mean (SD) C_{max} of the test product was 3.11 (1.06) µg/L with a median (IQR) T_{max} of 4.0 (2.6-7.5) hours. Mean (SD) C_{max} of the reference product was 2.91 (0.84) µg/L with a median (IQR) T_{max} of 6.0 (4.0-14.0) hours. Intrasubject coefficients of variation (derived from the mean square error of the ANOVA) were 10.2% (AUC_{0-72}) and 12.4% (C_{max}). A non-parametric Wilcoxon signed rank test demonstrated a significant difference in T_{max} (p=0,007).

Table 7.2 Pharmacokinetic parameters for amlodipine test (liquid) and reference (tablet) formulations after administration of a single 5-mg dose in 12 healthy adult volunteers.

	Test (liquid)		Reference (tablet)	
t_{max} (h)				
Median (IQR)	4.0	(2.6-7.5)	6.0	(4.0-14.0)
Range	2.00 - 10.0		2.50 - 23.8	
C_{max} (µg/L)				
Mean (SD)	3.11	(1.06)	2.91	(0.84)
Geometric mean (Geometric CV)	2.97	(30.8%)	2.80	(30.4%)
Range	2.07 - 5.83		1.80 - 4.54	
AUC_{0-72} (µg*h/L)				
Mean (SD)	102.7	(26.8)	108.2	(30.6)
Geometric mean (Geometric CV)	99.4	(27.7%)	104.2	(29.8%)
Range	57.83 - 141.44		60.94 - 168.10	
$AUC_{0-\infty}$ (µg*h/L)				
Mean (SD)	141.3	(50.3)	147.4	(49.6)
Geometric mean (Geometric CV)	133.7	(36.0%)	139.8	(35.3%)
Range	66.49 - 260.42		74.57 - 237.66	
$t_{1/2}$ (h)				
Mean (SD)	36.2	(10.9)	36.4	(8.95)
Range	25.2 - 66.1		25.2 - 52.6	

t_{max} = time to reach C_{max}; C_{max} = maximum plasma concentration; AUC_{0-72} = area under the concentration-time curve from time zero to 72 hours; $AUC_{0-\infty}$ = AUC from time zero to infinity; $t_{1/2}$ = terminal elimination half-life

Bioequivalence

The 90% confidence intervals of the test/reference ratios of the geometric means for the primary pharmacokinetic parameters AUC_{0-72} (88.24 - 104.37%) and C_{max} (99.00 - 121.40%) are within the acceptance range of 80.00-125.00% for bioequivalence, showing that the liquid is bioequivalent to the tablet.

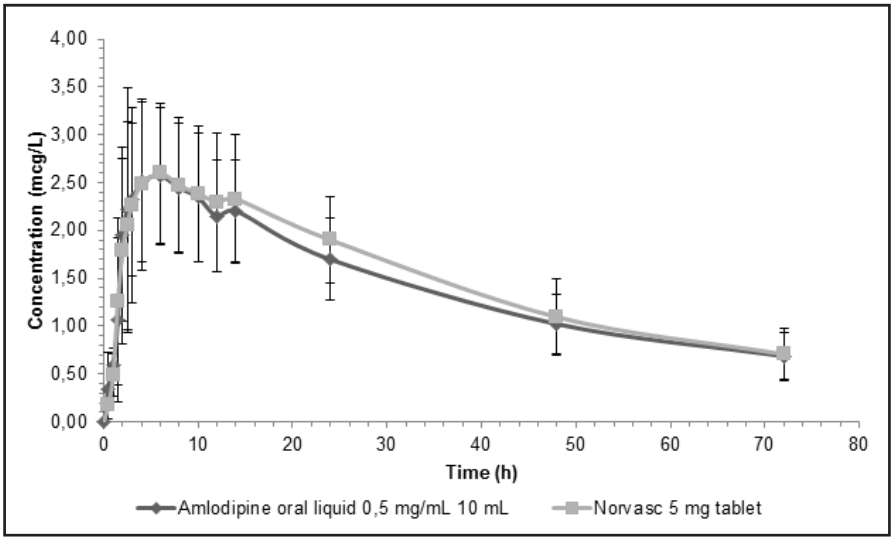

Figure 7.1 Composite (mean±SD) plasma concentration versus time curves after administration of a single 5-mg dose the test (liquid) and reference (tablet) formulations in healthy adult volunteers.

Taste assessment

The average first impression of the taste of the oral solution was rated between 'not good, not bad' and 'good' (mean 2.75 on 5 point scale where 1= very good and 5= very bad), with two subjects rating it as 'bad'. Two minutes after ingestion the taste of the oral solution was on average rated as 'good' (mean 2.17). Subjects described the taste as a combination

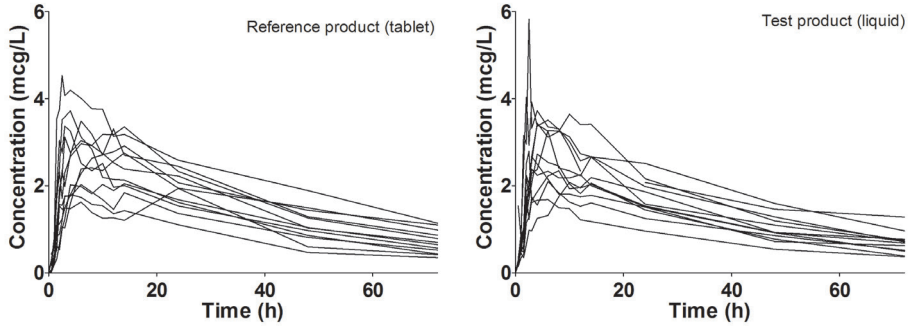

Figure 7.2 Individual amlodipine plasma concentration versus time plots of the liquid test formulation and the solid reference formulation.

of sweet and bitter. All of the subjects would take the oral solution again, if necessary. Only one subject would not recommend it to other people.

DISCUSSION

In this study a novel liquid formulation of amlodipine besylate was tested for bioequivalence to the reference Norvasc® tablet. The 90% confidence intervals of the ratios of the geometric means for the primary endpoints AUC_{0-72} and C_{max} were well within the pre-defined bioequivalence acceptance range of 80.00-125.00%. This means that the two formulations are bioequivalent according to EMA guidelines.

PK parameters for both formulations were similar, except for the anticipated shorter T_{max} observed for the liquid formulation. Since amlodipine is absorbed relatively slowly and no significant change in peak concentration occurs, this difference is deemed acceptable. The PK parameters were likewise comparable to previously published bioequivalence studies using Norvasc® 5 mg ($AUC_{0-\infty}$ mean 166.3, SD 76.7 µg*h/L (16) and $AUC_{0-\infty}$ mean 203.2, SD 52.1 µg*h/L (17)). The mean elimination half-life of 36 hours and its marked between subject variability found in this study are consistent with previous studies in young, healthy volunteers (10-12, 16, 18, 19). The individual half-lives in this study ranged between 25 and 66 hours, but the wash-out period between treatments was sufficiently large to achieve more than six half-lives for all subjects.

In both the mean concentration-time plots (Figure 7.1) and multiple individual concentration-time plots (Figure 7.2), a second peak was observed around t=12 hours. Other pharmacokinetic studies on amlodipine (10-13, 16, 18), did not show this profile, possibly due to more sparse blood sampling around that time point. However, in accordance with our results, a similar profile with a secondary peak was found in a study on the influence of gastrointestinal transit times on the AUC of several calcium antagonists, including amlodipine (20). It has been suggested that amlodipine undergoes enterohepatic circulation (21), which is supported by the excretion of metabolites in the feces (14). A possible explanation of the second peak could be re-entering of amlodipine in the intestinal tract with the excretion of bile during/after the evening meal.

Although the EMA guideline for the investigation of bioequivalence (9) recommends no intake of food for at least four hours post-dose, we limited the fasting period to one hour after the administration of the study drug, based upon several studies showing no direct influence of food on the absorption of amlodipine (22, 23). Likewise there was no restriction on water and tea intake as the dissolution of amlodipine from the dosage form is unlikely to effect the absorption (10, 21).

For small molecules, the EMA considers bioequivalence studies in healthy volunteers to be adequate to detect formulation differences and to allow for extrapolation of the results to populations for which the reference medicinal product is approved (9). It is generally accepted that gastro-intestinal permeability in children above the age of 2 years is equivalent to that observed in adults (24). For in vivo solubility however, there is debate as to whether results from bioequivalence studies can be directly extrapolated to pediatric patients (25), because of the relatively smaller volume of gastro-intestinal fluid in children. It is therefore desirable to further elucidate the pharmacokinetic performance of the amlodipine oral solution in the pediatric population. The results of this study will form the basis for a study protocol in children.

CONCLUSION

In conclusion, in this study we showed bioequivalence of the newly developed amlodipine oral solution compared to Norvasc® 5 mg tablets. With these results, the use of the liquid in the intended target population, children with chronic hypertension, can be safely explored in future studies.

ACKNOWLEDGEMENTS

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8

Amlodipine oral solution for the treatment of hypertension in children; population pharmacokinetics and acceptability study

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ABSTRACT

Aim

Amlodipine is a recommended antihypertensive agent for the treatment of paediatric hypertension of multiple aetiology, but the commercially available tablets and capsules are not suitable to treat young patients. This study aimed to characterize the pharmacokinetic properties (PK) of amlodipine, and to determine the association between patient characteristics and PK parameters in paediatric patients, using a population pharmacokinetic study design.

Methods

A population pharmacokinetic and acceptability study of an extemporaneous oral solution of 0.5 mg/ml was conducted in hypertensive children between the age of six months and 11 years. Population pharmacokinetic analysis was performed using NONMEM.

Results

Nine children with a median age of 2.9 years (IQR 1.8-8.4), receiving amlodipine chronically, with a median dose of 0.15 mg $kg^{-1}day^{-1}$ (IQR 0.11-0.18), were evaluable for analysis. Amlodipine pharmacokinetics were well described by a 1-compartment model with first-order absorption and elimination. The covariate analysis resulted in two significant covariates; weight was correlated with volume of distribution and sex was correlated with clearance. Based on the final model, clearance was reduced by approximately 30 % in females. Patient reported outcomes on taste from a five-point hedonic scale were available for five patients, who scored the taste from positive to slightly negative.

Conclusion

The results from the pharmacokinetic study and the acceptability assessment show that the amlodipine oral solution presented in this study offers an excellent treatment option for young paediatric patients requiring amlodipine treatment, with adequate pharmacokinetic properties and acceptability.

INTRODUCTION

Amlodipine is a long-acting dihydropyridine calcium channel blocker, and is a recommended antihypertensive agent for the treatment of paediatric hypertension of multiple aetiology, including before an underlying diagnosis is established (1). It is licensed for the treatment of patients from the age of six years, and pharmacokinetic and efficacy data for patients under the age of six years are limited. Pharmacokinetics of amlodipine are known to exhibit large interindividual variability. A previously published population pharmacokinetic (PK) model has demonstrated that young children (1 to <6 years) have significantly higher weight-normalized clearance values than older children and adults, but the data did not support a higher dose on a mg/kg basis, due to the enrolment of too few children below the age of 6 years (2).

For the treatment of young patients, the commercially available tablets of 5 and 10 mg are not sufficient to obtain the required dosages of 0.06-0.6 mg/kg per day (3, 4), and the solid dosage form may not be acceptable to many children. Amlodipine was therefore added to the 'Inventory of paediatric therapeutic needs' published by the European Medicines Agency (EMA), for need of an age-appropriate formulation and pharmacokinetic data for children especially below the age of 6 years (5).

In response to this therapeutic need, we designed and validated a 0.5 mg/ml amlodipine besylate paediatric oral solution, suitable for extemporaneous and batch scale compounding (6), and subsequently demonstrated bioequivalence of this liquid to 5 mg Norvasc tablets in healthy adult volunteers (7). Amlodipine is a typical biopharmaceutical classification system (BCS) class I drug, which means it is highly soluble in the gastrointestinal fluid, and highly permeable across the human intestinal membrane. As a result of a first-pass effect, bioavailability is variable (50-90%), but not influenced by food (8, 9). T_{max} values are also variable but generally long (4-14 h) (7). The slow arrival into the systemic circulation after oral administration is caused by passive transport across the intestinal membrane and excretion into the bile, rather than slow dissolution of the dosage form (10). The resulting gradual onset of action ensures that acute hypotension after oral administration is unlikely. In our bioequivalence study, a statistically significant, but not clinically relevant decrease in T_{max} from 6 to 4 hours was observed after administration of the oral solution compared to the originator tablets (7).

Considering the properties of amlodipine, it is unlikely that the oral solution will perform differently in paediatric patients compared to adults. Absorption from the oral solution might be slightly accelerated compared to a solid dosage form, but the overall exposure and therapeutic effect, should not be altered. The current study was therefore conducted to further characterize the pharmacokinetic properties of amlodipine, and to determine the association between patients' characteristics and the PK parameters, in patients aged 6 months to 12 years, using a population pharmacokinetic study design. Secondary endpoints were the acceptability of the amlodipine oral solution to the patients, the patient's or parents' preference of drug formulation, and the safety of the amlodipine oral solution.

METHODS

The study was designed as a prospective, open-label, multicenter clinical trial (NTR4623) in three university paediatric hospitals in The Netherlands. The study protocol was approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, The Netherlands. Written informed consent of the patient's parents or legal guardians was obtained prior to any study procedures.

Patients aged 6 months to 12 years were eligible for inclusion when they were treated with a stable dosage of amlodipine for the previous 2 weeks, and were expected to continue the same dosage for the duration of the study. Concurrent treatment with other antihypertensive medication or CYP3A4 affecting co-medication was permitted in a stable dosage. Exclusion criteria were transient, unstable, malignant, or accelerated hypertension, renal transplantation less than 4 months before inclusion or active nephrotic syndrome.

Upon inclusion, a trough blood level was collected, and study medication was provided in the patient's pre-existent dosage. Parents were asked to keep a medication diary with the time and date of study drug administration, and to note any missed doses. After a minimum of two weeks, to guarantee steady-state conditions, a study visit was scheduled at the hospital or at the patient's home. Three capillary blood samples were taken according to the blood sampling schedule in Table 8.1. When possible, an additional sample was taken for the measurement of creatinine and albumin.

Table 8.1 Blood sampling scheme during steady-state conditions.

	Dosing frequency	
	Once daily	Twice daily
Trough amlodipine level	T = 0 (prior to intake)	T = 0 (prior to intake)
During absorption phase	T = 2-6 hours	T = 2-6 hours
During elimination phase	T = 6-16 hours	T = 6-10 hours

The acceptability of the formulation was assessed by requesting parents to evaluate the use of the patient's regular amlodipine formulation and the study medication, using a questionnaire. At the study visit, children aged three years or older were requested to evaluate the taste of the oral solution, using a five-point hedonic scale combined with a visual analogue scale (Figure 8.1), and their preference for either formulation was obtained. VAS-scores were expressed as a percentage, and scores up to 60% (between neutral and slightly negative) were considered acceptable.

Active assessment of adverse events (AEs) based on known AEs related to amlodipine took place at inclusion and at the study visit. AEs were classified according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 of the U.S. Department of Health and Human Services (11). The electronic medical records of the patients were monitored for any additional AEs until seven days after cessation of the study medication.

The amlodipine (besylate) oral solution 0.5 mg/ml was developed by the Department of Hospital Pharmacy of the Erasmus Medical Center in collaboration with the Laboratory of Dutch Pharmacists, and is described in a separate publication (6). Study medication was dispensed with several appropriately sized oral syringes to accurately measure the amlodipine dose. The container was capped with an Adapta-cap (Baxter, Utrecht, NL) bottle adapter to fit the syringe. Amlodipine concentrations in plasma were measured using a validated ultra performance liquid chromatography tandem mass spectrometry (12). Blood samples collected at home visits were kept on ice and processed within 24 hours. Plasma samples were kept frozen at -80°C prior to analysis.

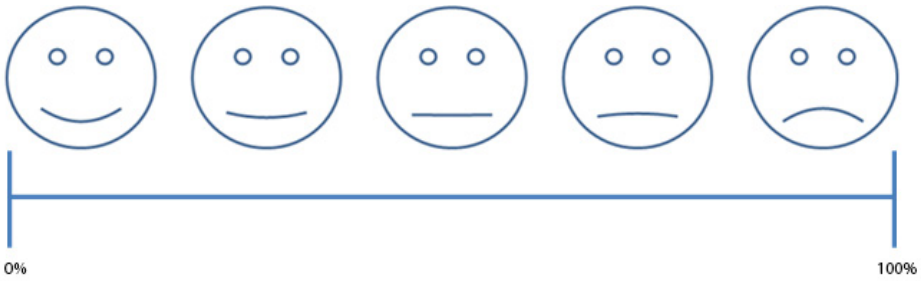

Figure 8.1 Five-point hedonic scale used in the taste assessments for patients three years and older.

Population PK Model Development

Population PK analysis was conducted using NONMEM version 7.2 (ICON Development Solutions, Ellicott City, MD, USA). The analysis was performed using logarithmically transformed concentrations and the FOCE method with INTERACTION. Tools used to evaluate and visualize the model were RStudio (version 0.98.1049), R (version 3.1.2), XPose (version 4.5.3) and PsN (version 4.6.0), all with the graphical interface Pirana (version 2.9.0). Model development consisted of three steps: 1) selection of a base model, 2) covariate analysis and 3) internal validation of the model. To determine the structural PK model, 1- and 2-compartment models were tested. Pharmacokinetic parameters were estimated in terms of apparent volume of distribution (Vd/F), apparent clearance (CL/F) and rate of absorption (ka). Residual variability between observed and predicted plasma concentrations was described using an additional error model for logarithmically transformed data. Model selection was based on the minimum value of objective function (OFV), parameter precision, shrinkage values, and visual inspection of the goodness-of-fit plots.

During the covariate analysis we evaluated if addition of covariates could explain interpatient variability (IPV). Covariates tested were bodyweight, age, sex and blood creatinine and albumin levels. Bodyweight was evaluated using allometric scaling with an exponential model and a fixed or estimated exponent. Addition of IPV, described using an exponential model, was evaluated for each PK parameter. Continuous covariates were normalised to the population median values and incorporated as power model functions (Equation 1), with the exception of weight that was normalised to the average adult

weight of 70 kg. Categorical covariates (sex) were transformed to binary covariates and incorporated as shown in Equation 2,

$$\theta_i = \theta_{pop} * \left(\frac{cov_i}{cov_m}\right)^{\theta cov} \quad (1)$$

$$\theta_i = \theta_{pop} * \theta_{cov}{}^{cov_i} \quad (2)$$

with θ_i being the individual model-predicted PK parameter (e.g. volume of distribution) for an individual with covariate value cov_i, θ_{pop} being the population estimate for that parameter, cov_m representing the median covariate value and θ_{cov} representing the covariate effect. In the equation for categorical covariates, cov_i is either 1 or 0.

Covariates were included using forward inclusion ($p<0.05$) and backward elimination ($p>0.01$). Additional criteria for the inclusion of covariates in the model were graphical evaluation of the parameter-covariate relationship and the decrease in parameter variability.

The final model was validated using visual predictive check (VPC) with 1000 simulated datasets, bootstrap analysis (n=1000) and sharkplots to evaluate the influence of covariates. Simulation for significant covariates was performed to describe the influence of the covariate on the PK.

Data analysis

Descriptive statistics were calculated using Windows Excel 2010 (Microsoft Corporation, Redmond, WA, USA).

RESULTS

A total of ten children were enrolled, nine of them completed the study. One child refused to take the study medication, and was excluded. The patient characteristics are displayed in Table 8.2 and individual parameters are displayed in Table 8.3. The majority of the patients had renal disease as underlying cause for hypertension (n=7), one patient had recently received a bone-marrow transplantation, and one patient had an unknown underlying cause. Six out of nine patients received amlodipine once daily. The medication diaries confirmed that all patients were adherent to the study medication.

Table 8.2 Patient characteristics (n=9).

Number of participants, n	9
Male gender, n (%)	6 (67%)
Age (years), median (IQR), range	2.9 (1.8-8.4), 1.2-9.9
Weight (kg), median (IQR), range	15.0 (9.9-26.8), 7.8-103
Amlodipine dose (mg $kg^{-1}day^{-1}$), median (IQR), range	0.15 (0.11-0.18), 0.10-0.22

Table 8.3 Individual patient characteristics (n=9).

Sex	Age (years)	Weight (kg)	Morning dose (mg)	Evening dose (mg)	Daily Dose (mg/kg)
m	1.2	9.2	1.0	0.0	0.11
m	1.8	7.8	0.5	0.5	0.13
f	1.9	12.0	2.5	0.0	0.21
f	2.9	14.0	1.2	1.2	0.17
m	2.9	15.0	1.0	1.0	0.13
m	8.1	23.0	5.0	0.0	0.22
f	8.2	26.0	2.5	0.0	0.10
m	8.8	103.0	0.0	15.0	0.15
m	9.9	29.0	5.0	0.0	0.17

m male; f female

Population pharmacokinetic model

The final dataset used in the NONMEM analysis consisted of thirty plasma concentrations from nine patients. Each patient had one concentration in the absorption phase available. The pharmacokinetic data were best described using a one-compartment model. The population estimate for Vd/F was 636 L (relative standard error [RSE] 27%), and the population estimate for CL/F was 12.4 L h^{-1} (RSE 8%). Shrinkage values were acceptable. Because of the low number of samples during the absorption phase, the population estimate of ka, 0.654 h^{-1}, was unprecise (RSE 72%), and thus no covariates were tested on ka. The covariate analysis resulted in two significant covariates; weight was correlated with Vd and sex was correlated with CL. No other covariates resulted in a significant

improvement of the model. The final model estimates are displayed in Table 8.4. Figure 8.2 shows that both the population predictions and individual predictions were evenly distributed around the line of identity when plotted against the observations. The bootstrap analysis of the final model showed that median parameter values as well as the 5th and 95th percentiles were in agreement with the model estimations and errors (Table 8.4). The visual predictive check indicated that the model adequately described the data. Shark plots showed that the covariates of the final model were not caused by a single individual.

Table 8.4 Parameter estimates of the base model, final model and bootstrap analysis.

	Structural model	Final Model			Bootstrap (n=1000)		
Parameter†	Point estimate		RSE (%)	Shrink-age (%)	Estimate	95% CI (lower)	95% CI (upper)
Ka (h^{-1})	0.582	0.654	72		0.65	0.05	2.03E+09
Vd/F (L)	345	636	27		622.77	127.65	6145.37
CL/F (L/h)	10.9	12.4	8		12.37	10.66	14.73
Covariate effect on Vd							
Weight		0.480	24		0.498	0.145	2.13
Covariate effect on Cl							
Sex‡		0.688	10		0.690	0.558	0.838
Between subject variability							
CL	23.5%	15.9%	21	1	14.5%	4.2%	21.3%
Residual variability	0.139	0.118	15	14	0.107	0.073	0.138

† $Vd/F\ (L) = 636*(WT/70)^{0.48}$; $CL/F\ (L/h) = 12.4*0.688^{sex}$
‡ 0 = male, 1 = female
Ka rate of absorption; Vd/F apparent volume of distribution; CL/F apparent clearance; RSE residual standard error;

Simulations

Based on the final model, clearance is reduced by approximately 30 % in females and volume of distribution is correlated with weight. The effect of sex and weight on the predicted plasma concentrations is shown in Figure 8.3, where steady state curves are simulated for patients of different weights and sex receiving a once daily dose of 2.5 mg. The simulations suggest that patients of the same sex, but a different weight, receive a similar exposure. Additionally, there is a marked difference in exposure between male and female patients.

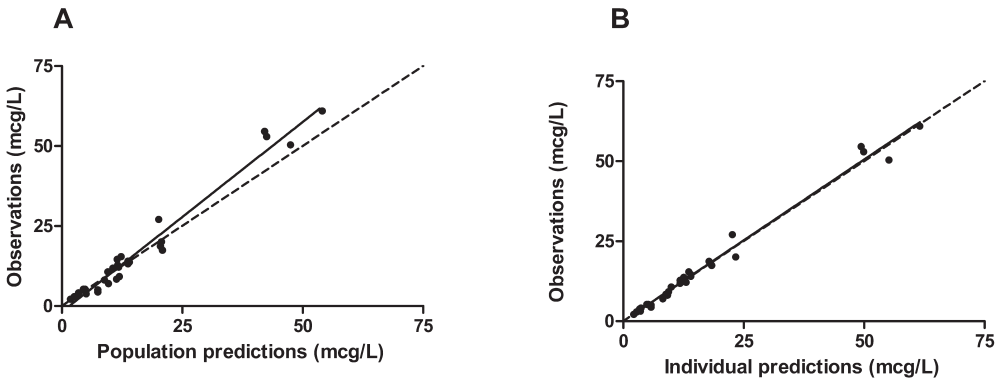

Figure 8.2 Goodness of fit plots for the final model. Population predictions vs. observations of amlodipine plasma concentrations (A) and individual predictions vs. observations of amlodipine plasma concentrations (B). The intermittent line represents the identity line, the solid line represents the line of best fit.

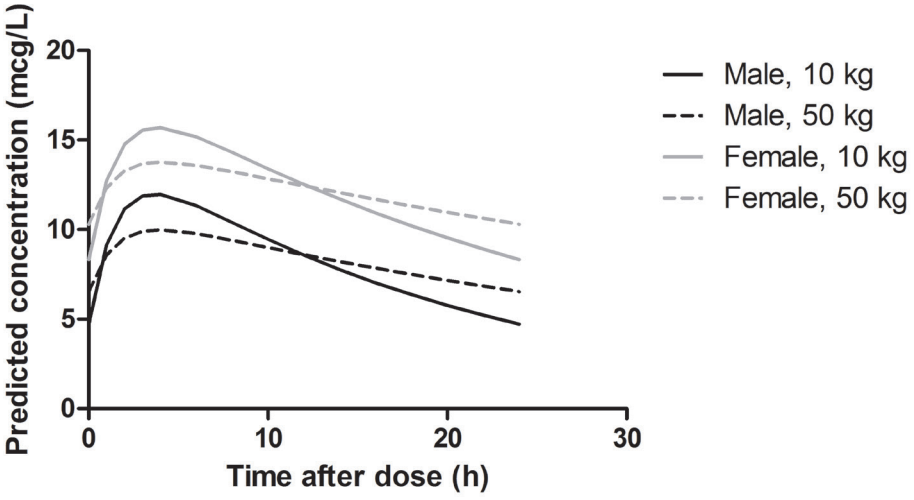

Figure 8.3 Simulated plasma profiles of 2,5 mg amlodipine dosed once daily during steady state for male and female patients of different weights.

Acceptability

The parents of four children preferred the study medication over the previous amlodipine formulation; three of these children previously received an extemporaneous suspension and one received tablets that needed to be halved and administered through a feeding tube. The parents of another four children preferred the previous amlodipine formulation over the study medication; twice because their child was capable of taking tablets, which was considered more convenient, and twice because the previous extemporaneous liquid formulation was more concentrated and a smaller volume could be administered. The parents of one child did not have any preference. Additionally, the parents of four children reported problems with either the Adapta-Cap (difficult to open, n=3), or the oral syringe (breaking of the tip n=1).

Five of the children were able to express a possible preference, three of them preferred their previous formulation (tablets (n=2), or 1 mg/ml solution (n=1)), one preferred the study medication over a suspension, and one did not have a preference. All of them indicated that taste was the main motivation for their preference. None of the parents reported to observe an aversion of their child towards the study medication, with the exception of the child that refused to take the study medication by mouth. The main reason for this was a general aversion for food, after having been fed through a feeding tube for several years prior to inclusion.

Patient reported outcomes were available for five patients. The patients scored taste on the VAS between 8 and 61% (slightly negative to positive), with a median of 51%. Scores up to 60% were considered acceptable.

Safety

None of the subjects had serious adverse events during the study period. Two subjects reported flushing, possibly related to amlodipine, before start of the study medication, but did not report it after treatment with the amlodipine oral solution. All other reported adverse events were judged to be unrelated to treatment with amlodipine. Peripheral oedema was not reported by any subject before or during the study.

DISCUSSION

This prospective population PK and acceptability study describes the pharmacokinetics of amlodipine in children and shows that the oral solution of amlodipine designed for young paediatric patients is acceptable to the target population.

The model constructed from the NONMEM analysis was able to predict the observed data with good precision. The covariates sex and bodyweight included in the model were able to explain 32% of the between subject variability. We did not observe a significant reduction in objective function when clearance was allometrically scaled, most likely due to the small dataset in combination with one large outlier. This resulted in a model where sex was a significant covariate on clearance, and bodyweight was a significant covariate on volume of distribution. The absence of allometric scaling means that our model predicts the same apparent clearance values for patients of different weights. Within our population of patients below the age of 10 years, these predictions are accurate, and the model was even able to accurately predict individual concentrations for our patient weighing 103 kg. It also means that predicted weight normalised clearance was higher in children with lower bodyweights, which was also observed by Flynn *et al. (2),* and who also constructed an alternative model without allometric scaling but with creatinine clearance as covariate on clearance.

The different age and weight distributions between the populations in our study and in the study by Flynn et al resulted in different parameter estimates for apparent clearance and apparent volume of distribution. More than halve of our population was below the age of three years, whereas Flynn et al included relatively few children below the age of six years. Parameter estimates were lower in our model, but for absorption rate constant and apparent volume of distribution not significantly different due to large confidence intervals in both models. A notable similarity between the two paediatric models was the correlation between sex and clearance, with an effect size of around 30%. This finding was not further discussed by Flynn *et al.*, but the effect on blood pressure was also observed in a large paediatric randomised clinical trial (13), and in paediatric patients with chronic kidney disease (14). Since a 30% difference in CL would justify an adjusted dosing schedule, further exploration of this observation is warranted. The estimate for absorption rate constant of 0.65 h^{-1} was unprecise, but lower than the estimate from Flynn *et al* of 0.80 h^{-1}, suggesting no increased absorption rate from the liquid compared to solid formulations. This finding confirms the observation in healthy adults that the oral solution is interchangeable with the tablets.

The acceptability assessment was also an important endpoint in this study. The taste assessment and questionnaire proved that the oral oral solution was well accepted, with four out of five patients reporting the taste to be neutral to positive. Taste and dose volume are the most commonly reported barriers to medicines administration in children (15), and are therefore important determinants of adherence. The dose volumes administered in this study were sometimes higher than those considered preferred by the EMA (≤5 ml for children up to 5 years, ≤10 ml for children up to 10 years) (16). This was reflected in some of the parents' preference for an extemporaneous, more concentrated amlodipine oral solution of 1.0 mg/ml. Our previous formulation studies have however shown that a 1.0 mg/ml solution has a decreased stability, with the precipitation of amlodipine as a result (6). When unnoticed, this could lead to serious under- or overdosing.

The results from this study are limited by the small number of subjects. The expected inclusion of 20 patients was not reached for several reasons. Firstly, for patients already using solid dosage forms, the prospect of having to switch to a liquid dosage form was often a reason not to participate. Secondly, for some parents, the study visit was considered too much of a burden. Despite these limitations, this study presents additional PK data from patients under the age of ten years, combined with an acceptability assessment of a high quality paediatric oral solution suitable for extemporaneous and batch scale compounding.

The results from the pharmacokinetic study and the acceptability assessment show that the amlodipine oral solution offers a treatment option for (younger) paediatric patients requiring amlodipine treatment, with adequate pharmacokinetic properties and acceptability.

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9

Oral lorazepam can be substituted for intravenous midazolam when weaning paediatric intensive care patients off sedation

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ABSTRACT

Aim

Intravenous sedatives used in the paediatric intensive care unit (PICU) need to be tapered after prolonged use to prevent iatrogenic withdrawal syndrome (IWS). We evaluated the occurrence of IWS and the levels of sedation before and after conversion from intravenous midazolam to oral lorazepam.

Methods

This was a retrospective, observational, single cohort study of children under the age of 18 admitted to the PICU of the Erasmus MC-Sophia Children's Hospital, Rotterdam, The Netherlands, between January 2013 and December 2014. The outcome parameters were the Sophia Observation withdrawal Symptoms (SOS) scale scores and COMFORT Behavior scale scores before and after conversion.

Results

Of the 79 patients who were weaned, 32 and 39 had before and after SOS scores and 77 had COMFORT scores. IWS was reported in 15/79 patients (19.0%) during the 48 hours before the start of lorazepam and 17/79 patients (21.5%) during the 48 hours after treatment started. Oversedation was seen in 16/79 patients (20.3%) during the 24 hours before substitution and in 30/79 patients (38.0%) during the 24 hours after substitution.

Conclusion

The weaning protocol was not able to prevent IWS in all patients, but converting from intravenous midazolam to oral lorazepam did not increase the incidence.

INTRODUCTION

Most children admitted to a paediatric intensive care unit (PICU) receive intravenous sedatives and analgesics to relieve anxiety, distress and pain and to tolerate mechanical ventilation and other PICU-related procedures. The most commonly used sedatives and analgesics in paediatrics are midazolam and opioids (1). Unfortunately, these drugs can cause iatrogenic withdrawal syndrome (IWS) after prolonged use (2).

To prevent IWS, a protocolled approach to taper the drugs and to regularly monitor withdrawal symptoms and sedation levels is recommended. Intravenously administered medication can be switched to oral dosage forms, to facilitate gradual weaning without the need for cardiorespiratory monitoring required for intravenous sedation, and to omit the need for intravenous access. Treatment can then be continued outside the PICU and removing intravenous access lowers the risk of infection. Oral lorazepam has a long half-life in children, with a median of 17 hours (range 8-53 hours), which prevents large fluctuations in plasma concentrations, and also has a lack of active metabolites. That is why it is often used off-label as a substitute for intravenous midazolam (2-4).

In our local weaning protocol, the calculation of the initial dose of oral lorazepam was based on a conversion factor proposed by Tobias *et al* (5), which assumed that lorazepam was twice as potent as midazolam and had a six-time longer half-life, based on adult data. This lorazepam starting dose is calculated irrespective of the potential impact of maturation of lorazepam and midazolam metabolism due to age or other factors influencing drug exposure, such as critical illness. In addition to this, the bioavailability of oral lorazepam in children is unknown and, therefore, no correction is possible for a potential incomplete bioavailability. In summary, this means that the current dosage of lorazepam for weaning of midazolam may not be optimal. At the time of our study, no clinical data on the conversion from midazolam to lorazepam in PICU settings was available in the literature.

Due to this limited information, the aim of this study was to evaluate the occurrence of IWS and the level of sedation before and after conversion from intravenous midazolam to oral lorazepam. We also wanted to assess the safety of our current midazolam to lorazepam conversion protocol.

METHODS

Design and study population

A retrospective, single centre, cohort study was performed to evaluate the move from intravenous midazolam to oral lorazepam to keep patients comfortable and prevent IWS. Our study population was admitted to the level five PICU of the Erasmus MC - Sophia Children's Hospital, Rotterdam, The Netherlands, between January 2013 and December 2014. Patients were selected from our Critical Care Suite electronic patient data management system (Picis Clinical Solutions SA, Barcelona, Spain), when they had received oral lorazepam following intravenous midazolam. The exclusion criteria were the use of midazolam and lorazepam for epilepsy or delirium, when the latter had been diagnosed by a trained psychiatrist, or for other reasons such as incidental sleep medication. The medical ethics committee of the hospital waived the need for institutional review board approval and informed consent according to the Dutch law on Medical Human Research.

IWS and sedation scores

To achieve optimal weaning, it is necessary to monitor symptoms of IWS from benzodiazepines and opioids and to monitor the level of sedation. These are assessed using the Sophia Observation withdrawal Symptoms (SOS) scale to determine IWS and the COMFORT behavior (COMFORT-B) scale to assess the level of sedation (6-8). The SOS scale consists of 15 items representing signs and symptoms of opioid and, or, benzodiazepine withdrawal, including changes in heart and respiratory rate and signs of discomfort. IWS scoring is initiated at start of weaning and performed at eight-hour intervals, when the occurrence of IWS is suspected, and to evaluate any interventions that were made to treat IWS. The COMFORT-B scale consists of six behavioural items and is applied in combination with the Nurses Interpretation of Sedation Score (NISS) (6) from the start of mechanical ventilation. It has been validated to assess the level of sedation in ventilated and non-ventilated children. Scoring is performed by the attending nurses at eight-hour intervals and if there are signs of distress or increasing discomfort. It continues until discharge from the PICU or until all sedative medication has been stopped.

Weaning protocol

Weaning of sedative and analgesic medication is initiated as soon as the patient's underlying condition and pathology improves, their electrolytes are within normal range and they are cardiovascularly stable. The protocol for weaning of continuous opioids and sedatives implemented at our PICU starts with decreasing continuous infusion rates of the drugs and the intervals depend on the preceding length of treatment. Infusion rates are decreased, one drug at a time, by 10% of the initial rate. This occurs every 24 hours when the patient has received the drug for 6-9 days and every 48 hours when they have received the drug for 10 days or more. The intravenous medication is converted to an effect-equivalent dose of oral medication within the same therapeutic class when the patient is due to be discharged to the general ward without cardiorespiratory monitoring, when intravenous access is no longer required or available or when prolonged weaning is expected. The initial daily dose of oral lorazepam is calculated by dividing the daily dose of midazolam by 12. This conversion is based on the lorazepam and midazolam ratio for half-life (6:1) and its relative potency (2:1) in adults (5). This lorazepam dose is administered orally four times a day and the intravenous midazolam is tapered over 24 hours as shown in Figure 9.1. Lorazepam is subsequently tapered in steps of 10% of the initial dose every 24 or 48 hours. If there are withdrawal symptoms, indicated by an SOS score of four or more, a rescue dose of 0.1 mg/kg midazolam is administered or the oral lorazepam dose is increased to the previous strength. If applicable, opioids and other sedatives, such as morphine, fentanyl, clonidine and pentobarbital, are also converted to oral alternatives in a similar manner, for example methadone, clonidine *per os* and phenobarbital, preferably with a minimum of 48 hours between conversions. They are tapered according to the same principles.

Medication

Intravenous midazolam was administered using a Perfusor FM syringe pump (B Braun Medical, Oss, The Netherlands), in concentrations of 1 mg/ml or 5 mg/ml dissolved in 5% glucose, which were prepared by the pharmacy. Oral midazolam for rescue administrations was available as an extemporaneous liquid of 1 mg/ml. Oral lorazepam was administered as either commercial tablets, extemporaneous capsules of 0.1 mg or a 4 mg/ml commercial

injection fluid that was administered orally. Solid dosage forms were usually dispersed in water and administered through a feeding tube.

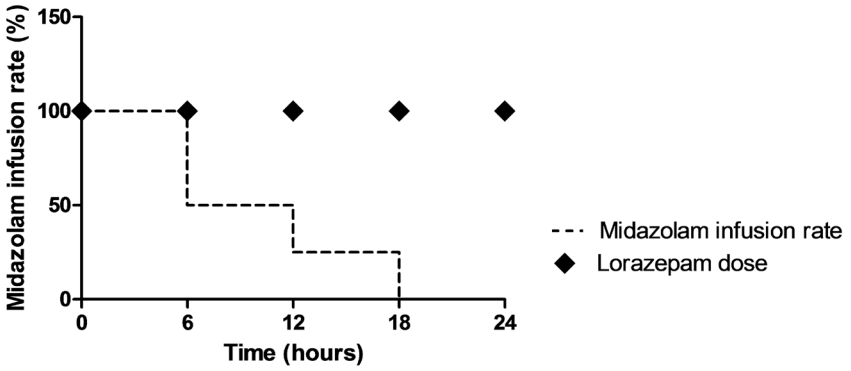

Figure 9.1 Tapering of midazolam after substitution with oral lorazepam. The intravenous midazolam dose is halved after the second administration of lorazepam, again halved after the 3rd administration of lorazepam and ceased after the 4th administration of lorazepam (24h after switch). The first dose of lorazepam is calculated upon the last infusion rate of midazolam.

Data collection

Data were extracted from the electronic medical records. The clinical and demographic parameters that were retrieved included age, sex, diagnosis, cumulative doses and duration of midazolam and lorazepam therapy, analgesic and sedative co-medication and the patient's destination after their discharge from the PICU.

Outcomes

The SOS scores were retrieved to determine the incidence of withdrawal from 48 hours before substitution to 48 hours after substitution. A cut-off score of at least four was defined as withdrawal. The COMFORT-B scores and NISS scores were analysed from 48 hours before substitution to 48 hours after substitution to determine the level of sedation. COMFORT-B scores of ≥23 or 11-22 with a NISS of one were regarded as undersedation, COMFORT-B scores of 11-22 with a NISS of two were regarded as adequate sedation and COMFORT-B scores of ≤10 or 11-22 with a NISS of three were regarded as oversedation. Similarly, the number of rescue dosages of midazolam and other sedatives were compared from 48 hours before to 48 hours after substitution. The frequency and severity of apnoeas and the need for flumazenil during the 48 hours after start of lorazepam were used to assess the safety of the conversion. Apnoeas were registered manually in the patient data management system by the attending physician or nurse as part of standard care. The agreement of the actual midazolam to lorazepam conversion with the conversion protocol was assessed with respect to the dose calculation of lorazepam and the tapering of midazolam within 24 hours after conversion.

Analysis

Data were analysed using IBM SPSS statistics version 21.0 (IBM Corporation, New York,

USA). Demographic and clinical data were processed using descriptive statistics. The number of rescue administrations of midazolam and other sedatives before and after substitution were compared using a paired-sample t-test.

RESULTS

During the 24-month study period between January 2013 and December 2014, 111 cases met the inclusion criterion for oral lorazepam use after intravenous midazolam therapy. After excluding three patients who started lorazepam in 2012, 20 patients who received lorazepam for other purposes than weaning, and excluding multiple occasions within one subject (n=9), 79 cases were included for further analysis. The patient characteristics are listed in Table 9.1.

Table 9.1 Patient characteristics (n=79).

Parameter		
Sex:	n	%
Male	37	46.8
Female	42	53.2
Age median (months) (IQR)	5.3 (1.7-19.8)	
Age:	n	%
0-27 days	13	16.5
28 days -11 months	40	50.6
12- 23 months	8	10.1
2-11 years	16	20.2
12-18 years	2	2.5
Weight median (kg) (IQR)	5.5 (3.6-10.0)	
Reason for PICU admission:	n	%
Cardiac	30	28.0
Non-cardiac surgical	4	5.1
Neurological	1	1.3
Infection/respiratory	19	24.1
Trauma	2	2.5
Congenital	9	11.4
Other	14	17.7
Ventilation	79	100
ECMO therapy	7	8.9
Transfer after PICU:	n	%
Home	7	8.9
Other hospital	18	22.8
Other department	45	57.0
Mortality	9	11.4
Median length of PICU stay		
Days (range)	32 (4-183)	

ECMO = extracorporeal membrane oxygenation; IQR = inter quartile range; PICU = paediatric intensive care unit

At the point of the midazolam to lorazepam switch, the median duration of midazolam infusion, from the day of admittance to the Sophia Children's Hospital, was 12 days (range 1-69) and the median cumulative dose was 46.5 mg/kg (range 0.47-287). We also noted that 23 patients were still on invasive ventilation and 11 patients had received midazolam at infusion rates that were higher than 0.35 mg/kg/h during their admission. Further information on the patients' sedative treatment during PICU admission is summarised in Table 9.2.

Table 9.2 Sedative treatment characteristics during PICU admission (n=79).

Parameter	**Median (range)**	**unit**
Median dose per patient:		
Midazolam[a]	130 (30-393)	mcg/kg/h
Lorazepam[b]	0.30 (0.08-2.76)	mg/kg/d
Cumulative dose:		
Midazolam[c]	46.5 (0.47-287)	mg/kg
Lorazepam	1.42 (0.08-79.32)	mg/kg
Maximum infusion rate before subsitution:		
Midazolam	300 (12-1000)	mcg/kg/h
Duration of infusion until substitution:		
Midazolam	12 (1-69)	days
Duration of midazolam therapy until substitution[d]	**n**	**%**
< 5 days	3	3.8
5-10 days	16	20.3
> 10 days	60	75.9
Duration of lorazepam taper:	**Days**	**(range)**
Lorazepam[e]	22 (3-97)	(n=45)
Fixed-interval and continuous sedative and analgesic co-medication:	**n**	**%**
Alimemazine *po*	10	13
Clonidine		
iv	41	52
po	23	29
Esketamine *iv*	26	33
Fentanyl *iv*	9	11
Methadone *po*	16	20
Morphine *iv*	73	92
Pentobarbital *iv*	3	4
Propofol *iv*	19	24

[a] Throughout PICU admission, [b] Starting dose at substitution, [c] Until substitution, [d] Midazolam therapy was calculated from the first administration to the last administration in the Sophia Children's hospital. The short administration of one day is due to the transfer from another hospital. [e] n=45. Total lorazepam duration, including use at home. Only the patients with complete post clinical duration were used to calculate the median.
po = orally; iv = intravenous; PICU = paediatric intensive care unit.

The SOS scores were available for 32/79 (40.5%) of the patients in the 48 hours before substitution and 39/79 (49.4%) of the patients in the 48 hours after substitution. The median score per patient before the start of lorazepam ranged between 0-9.0, with 15 patients (19.0%) having one or more SOS score of at least four, indicating IWS. After the start of lorazepam the median score per patient ranged between 0-5.0, with 17 patients (21.5%) having one or more SOS score of at least four. In eight of these 17 patients, the morphine infusion rates were decreased during the 96 hours around conversion. Figure 9.2 shows the range of the highest SOS score per patient within our study period. Seven patients experienced IWS both before and after substitution and 11 patients experienced both oversedation and IWS in the 48 hours after substitution.

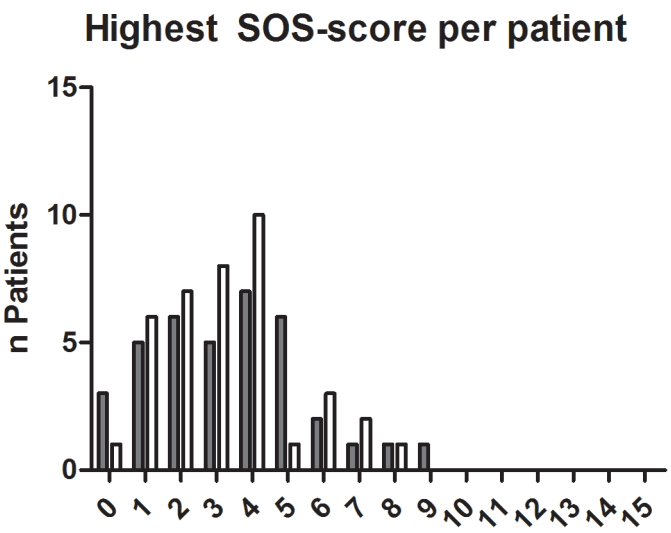

Figure 9.2 Distribution of the highest SOS score per patient during the first 48 hours before substitution (grey bars) and 48 hours after substation (open bars) of iv midazolam with oral lorazepam. Maximum score is 15, with scores ≥ 4 indicating withdrawal.

COMFORT-B scores were available for 77/79 patients (97.5%). All the available scores are shown in Figure 9.3, with a median of three scores per patient per day. From a total of 1,122 COMFORT-B scores, 136 incidences of oversedation and 150 incidences of undersedation were determined, in combination with the NISS, during the 96-hour study period. Only 44 of the incidences of undersedation were accounted for by COMFORT-B scores of at least 23 and the other 106 by a COMFORT-B score between 11-22 and a NISS of one.

In some patients the COMFORT-B scores, in combination with the NISS, were outside the adequate sedation range and these are presented in Figure 9.4. This figure shows that the incidence of oversedation increased after substitution with lorazepam. During the two days before substitution, 13 and 16 patients, respectively, experienced oversedation compared to 39 and 30 patients in the two days after substitution. Undersedation decreased from 28 and 21 patients before lorazepam initiation to 16 and 13 patients after the start of lorazepam.

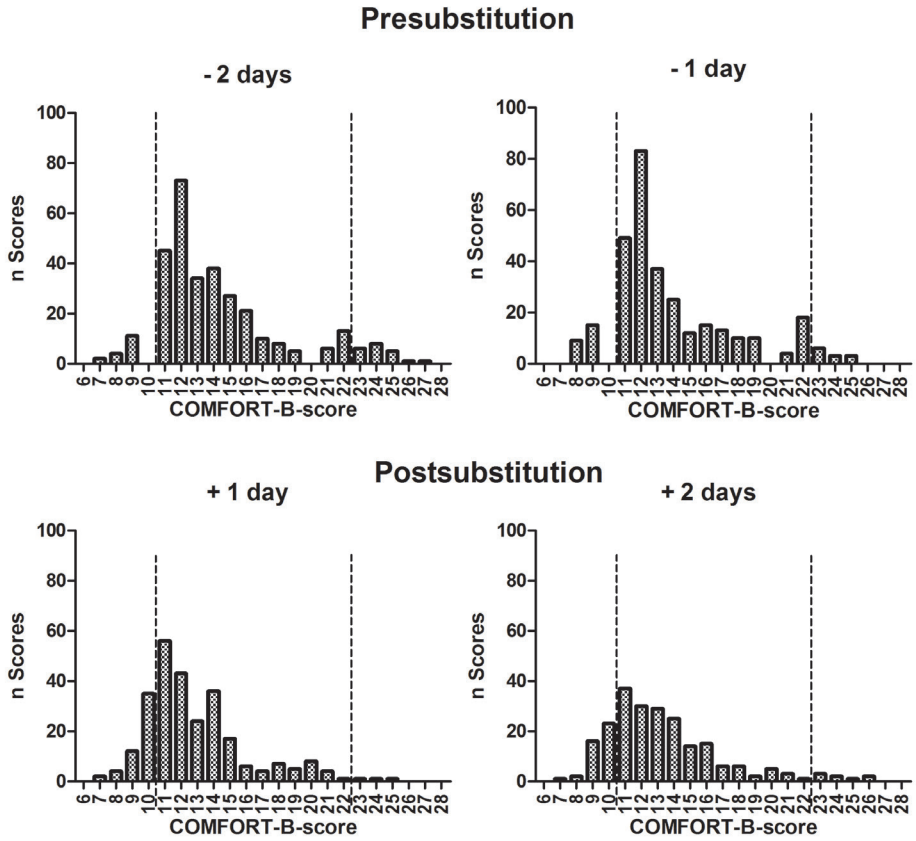

Figure 9.3 Histograms of available COMFORT-B scores during the four different study periods. The window between the dotted lines show scores that are regarded as adequate sedation, while lower scores (≤10) are regarded as oversedation and higher scores (≥23) as undersedation.

A total of 34 patients (43.0%) received one or more rescue administrations of midazolam before substitution, compared to 19 patients (24.1%) after substitution, with a 95% confidence interval (95% CI) of -0.06-0.77, p=0.096. Furthermore, 29 patients (36.7%) received rescue administrations of other sedatives before substitution compared to 21 patients (26.6%) after substitution (95% CI -0.18-0.94, p=0.178). In total, 50 patients (63.3%) received rescue administrations before substitution and 34 patients (43.0%) after substitution with a median of two administrations in both periods. During the 48-hour post substitution period, 56 patients (70.9%) continued their sedative or analgesic co-medication. Co-medication was decreased in 44 patients and increased in three patients.

Regarding the safety of the substitution, no apnoeas were reported and no flumazenil was prescribed during the 96 hours around the conversion.

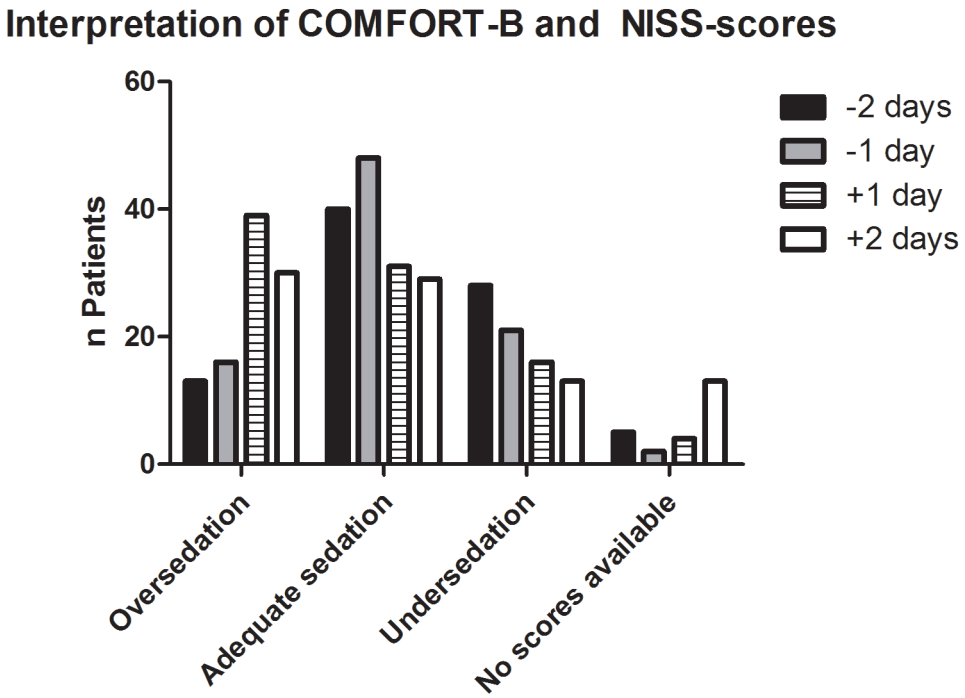

Figure 9.4 Oversedation: COMFORT-B scores ≤10 or 11-22 with NISS=3. Adequate sedation: COMFORT-B scores of 11-22 with NISS =2. Undersedation: COMFORT-B scores ≥23 or 11-22 with NISS =1. For study periods 1-4 respectively 7, 9, 11 and 6 children were both under- and oversedated.

Adherence to the conversion protocol was variable. The median midazolam/lorazepam dose ratio was 11.4 (range 1.31-22.6) and 62.0% of the ratios were between 10 and 14. In 45.6% of the patients, midazolam was tapered in a timeframe of 24 hours from substitution, in agreement with the protocol. In 32.9%, intravenous midazolam was discontinued before 24 hours and in 21.5%, simultaneous administration of intravenous midazolam and oral lorazepam continued for more than 24 hours.

DISCUSSION

Our midazolam to lorazepam switch protocol to prevent IWS appeared to be effective in the majority of patients, as no increase in the occurrence of IWS was detected. Nevertheless, at least 20% of patients still experienced withdrawal symptoms, while almost 40% showed signs of oversedation in the early stages after conversion.

Based upon the available SOS scores, the incidence of IWS was similar before and after conversion to lorazepam. A limitation is that only about half of the patients were scored for withdrawal, making the results hard to extrapolate. When we assume that the exhibition of IWS symptoms is a trigger to start collecting SOS scores, the absence of SOS scores may be seen as a sign that the patients were doing well, but this needs to be verified in a prospective setting. Furthermore, the SOS scale cannot discriminate between opioid and benzodiazepine withdrawal. This means that the reported IWS cannot unequivocally be

attributed to benzodiazepine withdrawal, especially in the eight patients where morphine was tapered simultaneously. Nevertheless, we did not observe an increase in IWS after the conversion to lorazepam.

The incidence of IWS in critically ill children has been reported to range from 13%-87% (8-19). This large variation was the result of small sample sizes, a large variety in often unvalidated assessment methods and non-standardised or absent sedation protocols and weaning regimens. Identified risk factors for IWS are cumulative doses of midazolam greater than 40 mg/kg (8, 11), infusion of opioids and benzodiazepines for more than five days (8, 11, 13), and midazolam infusion rates above 0.35-0.42 mg/kg/h (18, 19). Taking into consideration the clinical patient characteristics, such as the high cumulative doses of midazolam and long PICU stays, it becomes apparent that the patients in our cohort were at high risk for developing IWS. In our retrospective cohort, based upon the available SOS scores, IWS was diagnosed in one-fifth of the patients, both before and after substitution.

The majority of the collected COMFORT-B scores were within the target range for adequate sedation, with a tendency towards more oversedation post-substitution. This could suggest supratherapeutic dosages of sedatives, especially during the first 24 hours in which midazolam and lorazepam were simultaneously administered. To put these findings into perspective, COMFORT-B scores of nine and 10 could be the result of a comfortably asleep child with normal muscle and facial tone and is not necessarily indicative of an unsafe situation. Considering it may take a number of days to reach steady-state plasma levels of lorazepam due to its long half-life, it seems rational to start with lorazepam while phasing out midazolam to ensure adequate exposure. The absence of apnoeas and flumazenil administration during the study period provides evidence that the combined blood levels of benzodiazepines were not within the toxic range. It is notable that several patients experienced both oversedation and withdrawal after substitution, which illustrates the complexity of managing IWS. The comparison of rescue administrations of midazolam and other sedatives yielded no statistically significant results.

The lorazepam dose calculation was based on the relative half-life and potency of lorazepam versus midazolam, as determined in adult patients, and irrespective of individual patient characteristics. Lorazepam is primarily metabolised through conjugation with glucuronic acid by multiple hepatic UDP-glucuronosyltransferase enzymes, to inactive metabolites. The maturation rates of involved enzyme systems differ between the subtypes, but may well extend beyond the age of two years, based upon gene expression data and *in vivo* experiments (20, 21). Paediatric pharmacokinetic data after the oral administration of lorazepam are unavailable. At the moment, there are insufficient data available to establish an age-dependent conversion factor. Midazolam pharmacokinetics in paediatric patients are well studied and are highly dependent on CYP3A4 activity. High blood levels of midazolam might be caused by delayed clearance due to immature metabolism at a neonatal age (22), ongoing inflammation and critical illness (23), co-medication, accumulation of its active metabolites after prolonged use (19) or renal insufficiency (24). None of these factors are currently considered in the dose calculation.

This retrospective analysis of a weaning strategy reflects clinical practice in patients in a complex, intensive care setting. We acknowledge that our study had several limitations. Although COMFORT-B scores were taken regularly, we found that SOS scores were underreported. In addition, the lorazepam dose calculation in some patients was based upon the midazolam dosage rate at the moment of conversion instead of the

cumulative dose of the last 24 hours, resulting in different dosing strategies. Since 2017, a lorazepam extemporaneous oral liquid of 1 mg/ml has been available (25). As a result, oral administration of injection fluid is no longer applied and capsules are no longer used. The dose conversion is now checked by the attending pharmacist. One further limitation was that the concomitant use of other central nervous depressants was common during PICU stays in our study and this hindered the attribution of the observations to the conversion from midazolam to lorazepam.

In the past two decades, considerable progress has been made in recognising the need for weaning-off sedation strategies in PICUs. Risk factors for the development of IWS have been identified and scoring systems have been validated and implemented to monitor the patients. This study was the first to specifically address the use of oral lorazepam in the weaning-off sedation strategy in PICU patients.

CONCLUSION

The weaning protocol for sedatives using lorazepam did not increase the incidence of IWS and appeared to be safe. A better understanding of the factors that explain variations in both pharmacokinetics and pharmacodynamics may help us to further tailor weaning strategies to the individual patient.

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10

Bioavailability of a pediatric lorazepam oral liquid in pediatric intensive care patients

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Manuscript in preparation

ABSTRACT

Objective

Lorazepam is commonly administered to pediatric patients in several off-label indications. We recently developed an extemporaneous pediatric oral solution as existing formulations are not appropriate for young children. The aim of this study was to estimate oral bioavailability of this newly developed formulation in pediatric intensive care patients, receiving lorazepam for the prevention of iatrogenic withdrawal syndrome, using a population pharmacokinetic approach.

Design

A prospective, single-center, population pharmacokinetic study.

Setting

The pediatric intensive care unit of the Sophia Children's Hospital, Rotterdam, The Netherlands.

Patients

Pediatric intensive care patients aged up to 11 years, receiving lorazepam for the prevention of iatrogenic withdrawal syndrome.

Interventions

Plasma concentrations were collected after intravenous and oral administration, using a sparse sampling approach.

Measurements

Data were analyzed using non-linear mixed effect modelling.

Main Results

Eight patients, with a median age (IQR) of 0.49 (0.15-7.98) years, provided blood samples for the pharmacokinetic analysis. A one-compartment model was developed and bioavailability was estimated at 80% (bootstrap 95% confidence interval 59-96%). Parameter estimates for a subject of 70 kg indicated values of lorazepam clearance of 7.43 L h^{-1} (interpatient variability 32.1%), and a volume of distribution of 82.6 L. The exponent for allometric scaling was fixed at 0.75 for clearance and 1 for the volume of distribution. The covariate analysis did not result in significant covariates.

Conclusion

The lorazepam pediatric oral liquid demonstrated high oral bioavailability. Our estimate for clearance and volume of distribution in combination with previously reported values, indicate that a four times daily dosing schedule is necessary to maintain stable plasma levels of lorazepam.

INTRODUCTION

Lorazepam (7-chloro-5-(2-chlorophenyl)-3-hydroxy-2,3-dihydro-1H-1,4-benzodiazepin-2-one) is a benzodiazepine that is regularly administered to pediatric patients. The intravenous injection fluid is registered for preprocedural sedation in patients from the age of 12 years, but it is also applied in several off-label indications, for instance the emergent treatment of status epilepticus (1). In the pediatric intensive care unit (PICU), orally administered lorazepam is used to prevent iatrogenic withdrawal syndrome (IWS) after prolonged administration of intravenous benzodiazepines (2).

To facilitate gradual weaning of benzodiazepines, intravenously administered midazolam is switched to orally administered lorazepam in an equivalent dose. Lorazepam is the benzodiazepine of choice because of its long half-life, hepatic elimination, and lack of active metabolites. For patients being converted from intravenous midazolam, the initial daily dose of oral lorazepam is calculated by dividing the preceding daily dose of midazolam by 12. This conversion was based on the lorazepam and midazolam ratio for half-life (6:1) and its relative potency (2:1) in adults, proposed by JD Tobias (3). To avoid high peak concentrations and retain steady exposure, the daily dose is divided over four or more administrations.

For the indication of gradual weaning, the commercially available dosage forms of lorazepam, injection fluid and tablets, are usually not appropriate. The injection fluid can be administered orally, but contains high amounts of possibly harmful excipients, namely propylene glycol and benzyl alcohol, and the tablets do not provide flexible dosing. Capsules can be compounded extemporaneously in the dosage needed, but they need to be dissolved before administration to young patients and are difficult to administer through feeding tubes. For this reason, a lorazepam extemporaneous oral liquid of 1 mg/ml was developed, with adequate stability, taste and dosing flexibility (4).

The population pharmacokinetics of lorazepam after intravenous administration in pediatric patients from the age of three months with status epilepticus were successfully described by Gonzales *et al* (5), using a two-compartment model. They found that, after accounting for body weight, age was a statistically significant covariate, with a modest impact on weight normalized clearance, likely caused by developmental differences in UGT2B7 activity (6). Post hoc elimination half-life estimates ranged from 8.2 to 53.6 hours. Further pharmacokinetic data come from studies in pediatric patients with severe malaria convulsions (7), critically ill neonates with seizures (8), and outpatient pediatric patients treated for acute lymphocytic leukemia (9, 10), but are limited to intravenous or intramuscular single doses. Therefore, no pediatric pharmacokinetic data are available on repeated dose or orally administered lorazepam.

From adult non-compartmental pharmacokinetic studies, it was established that lorazepam shows rapid and almost complete (>90%) oral absorption after a lag time of around 15 minutes, probably attributable to the time needed for dissolution of the solid dosage form and gastric emptying into the site of absorption. Peak concentrations are usually reached within 2.5 hours, and the apparent volume of distribution (Vd) is only slightly larger than body weight. The terminal elimination half-life is on average 12-15 hours, and conjugation with glucuronide is the major path of elimination. Repeated dose administration has shown that lorazepam displays linear pharmacokinetics and does not induce or inhibit its own metabolism (11, 12).

From the biopharmaceutical characteristics and physical-chemical properties of lorazepam, it is unlikely that drug absorption will differ significantly in pediatric patients compared to adults. The absence of both active transport across the intestinal wall and a significant first-pass effect do not give reason to expect a decrease in absorption (11). Independent from age, the lag time to absorption will likely be reduced with the administration of an oral solution, as no dissolution is needed, and administration directly into the duodenum in patients with a naso-duodenal feeding tube will further reduce this lag time.

Since the prevention of IWS requires adequate and stable exposure, a correct initial dose calculation after conversion from midazolam is required. For this reason, we wanted to confirm the hypothesis that oral bioavailability of lorazepam liquid is similar in pediatric intensive care patients compared to values reported in adults. Since this vulnerable population does not allow for a classical non-compartmental pharmacokinetics study design with a dense sampling schedule, the primary objective of this study was to determine the oral bioavailability of the lorazepam oral solution 1 mg/ml in the pediatric ICU population, in patients aged up to 11 years, using a population pharmacokinetic approach. Secondary objectives were to explore the impact of age, sex and bodyweight on pharmacokinetic parameters, to assess the occurrence of iatrogenic withdrawal syndrome, to assess the occurrence of over- and undersedation, and to assess the acceptability and safety of the lorazepam liquid formulation in the target population.

METHODS

We conducted a prospective, single-center clinical trial (NTR5112) to evaluate the bioavailability and pharmacokinetics of a 1 mg/ml lorazepam oral solution (4). Patients were enrolled at the pediatric intensive care unit of the Erasmus MC-Sophia Children's Hospital, Rotterdam, The Netherlands. The study was approved by the local medical ethics committee (ref. no. MEC 2015-290). Written informed consent of the patient's parents or legal guardians was obtained prior to any study procedures.

Patients were eligible for inclusion if they were aged up to 11 years and were receiving lorazepam or scheduled to start lorazepam for clinical purposes. Exclusion criteria were concomitant treatment with another investigational drug and contraindications for lorazepam use: severe liver insufficiency, defined as five times the upper level of ALAT and ASAT; hypersensitivity to lorazepam; and myasthenia gravis.

Patients were treated according to the local protocol for weaning of opioids and sedatives (2). Lorazepam was administered four to six times daily, with a maximum of 3 mg per single dose. Intravenous midazolam was tapered over 24 hours as shown in Figure 10.1. To enable the determination of oral bioavailability, patients who were not already receiving lorazepam were given one single intravenous dose (Temesta® IV fluid) via an existing intravenous access at 50% of the calculated oral dose as a replacement of the first oral lorazepam dose. Blood samples of 0.5 ml were taken three times within the same dosing interval for each administration route, at variable time points, using an existing arterial line or during venous or capillary blood sampling for clinical purposes. Patients already receiving lorazepam at the moment of inclusion were sampled during steady state conditions. Blood was collected in 0.5 ml BD Microtainer® K2E Tubes (Becton, Dickinson and Company, Plymouth, United Kingdom), plasma was separated within the same day and frozen at -80°C until analysis. All study medication was prepared by the pharmacy. Lorazepam plasma concentrations were determined at the ISO15189 certified laboratory of the Clinical Pharmacy and Pharmacology department of the University Medical Center

Groningen, using a validated LC-MS/MS method.

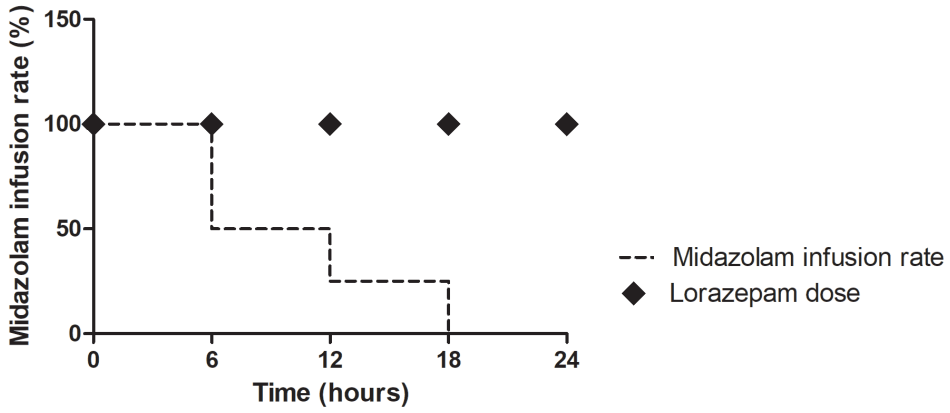

Figure 10.1 Intravenous midazolam to oral lorazepam conversion. The intravenous midazolam dose is halved after the second administration of lorazepam, again halved after the 3rd administration of lorazepam and ceased after the 4th administration of lorazepam (24h after conversion). The dose of lorazepam is calculated by dividing the 24h cumulative dose of midazolam by 12.

Pharmacokinetic analysis

Population pharmacokinetic analysis was conducted using NONMEM version 7.2 (ICON Development Solutions, Ellicott City, MD, USA). The analysis was performed using the FOCE method with INTERACTION and the ADVAN5 and TRANS1 subroutine. Tools used to evaluate and visualize the model were RStudio (version 0.98.1049), R (version 3.1.2), XPose (version 4.5.3) and PsN (version 4.6.0), all with the graphical interface Pirana (version 2.9.0). Model development consisted of three steps: 1) selection of a base model, 2) covariate analysis and 3) internal validation of the model. To determine the structural pharmacokinetic model, 1- and 2-compartment models were tested. The base model included allometric scaling using total body weight (WT) to account for size differences before consideration of other covariates. Pharmacokinetic parameters were estimated in terms of bioavailability (F), volume of distribution (Vd), clearance (CL) and rate of absorption (ka). Addition of inter patient variability (IPV), described using an exponential model, was evaluated for each pharmacokinetic parameter. Residual variability between observed and predicted plasma concentrations was described using an proportional error model. Model selection was based on the minimum value of objective function (OFV), parameter estimates and goodness-of-fit plots.

During the covariate analysis we evaluated if addition of covariates could explain interpatient variability (IPV). Covariates tested were body weight (estimation of exponent for allometric scaling), age and sex. Continuous covariates were normalized to the population median values and incorporated as power model functions (Equation 1), with the exception of body weight which was normalized to the average adult body weight of 70 kg. Categorical covariates were transformed to binary covariates and incorporated as

shown in Equation 2,

$$\theta_i = \theta_{pop} * \left(\frac{cov_i}{cov_m}\right)^{\theta cov} \quad (1)$$

$$\theta_i = \theta_{pop} * \theta_{cov}{}^{cov_i} \quad (2)$$

with θ_i being the individual model-predicted pharmacokinetic parameter (e.g. volume of distribution) for an individual with covariate value cov_i, θ_{pop} being the population estimate for that parameter, cov_m representing the median covariate value and θ_{cov} representing the covariate effect. In the equation for categorical covariates, cov_i is either 1 or 0.

Covariates were included using forward inclusion ($p<0.05$) and backward elimination ($p>0.01$). Additional criteria for the inclusion of covariates in the model were graphical evaluation of the parameter-covariate relationship and the decrease in parameter variability.

The final model was validated using visual predictive check (VPC) with 1000 simulated datasets, and bootstrap analysis (n=1000).

Secondary endpoints

IWS and sedation scores were collected during the pharmacokinetic study. As part of standard of care, the occurrence of IWS was assessed using the Sophia Observation withdrawal Symptoms (SOS) scale and the level of sedation was monitored using the COMFORT behavior (COMFORT-B) scale in combination with the Nurses Interpretation of Sedation Score (NISS) (13, 14). SOS scores ≥4 were regarded as a sign of IWS. COMFORT-B scores of ≥23 or 11-22 with a NISS of one were regarded as undersedation, COMFORT-B scores of 11-22 with a NISS of two were regarded as adequate sedation and COMFORT-B scores of ≤10 or 11-22 with a NISS of three were regarded as oversedation. The need for flumazenil during the pharmacokinetic study was used to assess the safety of the lorazepam liquid. Serious adverse events were monitored for as long as the patient was using study medication, which could be administered until discharge from the hospital. An acceptability assessment using a five point hedonic scale was part of the study protocol, but since all patients received the study drug through a feeding tube, no assessment was performed.

RESULTS

Eight patients admitted to the intensive care unit between December 2015 and December 2017 were included and provided blood samples for the pharmacokinetic analysis, collected over a period between one and seven days. Patient characteristics are displayed in Table 10.1. Noteworthy is that the age and sex distribution was not balanced, with five boys under the age of one year, and three girls around eight years old. From five patients samples could be collected after both intravenous and oral administration of lorazepam, from two patients only after oral administration, and from one patient only after intravenous administration. All patients had one or more feeding tubes in place, Table 10.1 states the type of tube used for study drug administration. Figure 10.2 displays the observed plasma concentrations after oral administration per individual patient.

Table 10.1 Patient characteristics.

Clinical characteristics at inclusion	n = 8
Age (years), median (IQR), range	0.49 (0.15-7.98), 0.08-8.67
Weight (kg), median (IQR), range	6.95 (4.5-19.3), 3.2-29.7
Male gender, n (%)	5 (62.5%)
Reason admission PICU	
Respiratory disorder	3
Cardiac disorder	5
Respiratory support at start of pharmacokinetic sampling	
Invasive	3
Non-invasive	5
Lorazepam daily oral dose (mg $kg^{-1}day^{-1}$), median (IQR), range	0.29 (0.23-0.41), 0.17-0.51
Lorazepam single oral dose (mg kg^{-1}), median (IQR), range	0.07 (0.06-0.10), 0.05-0.13
Administration of oral lorazepam (n=7)	
Naso-gastric administration	4 (50%)
Naso-duodenal administration	3 (37.5%)

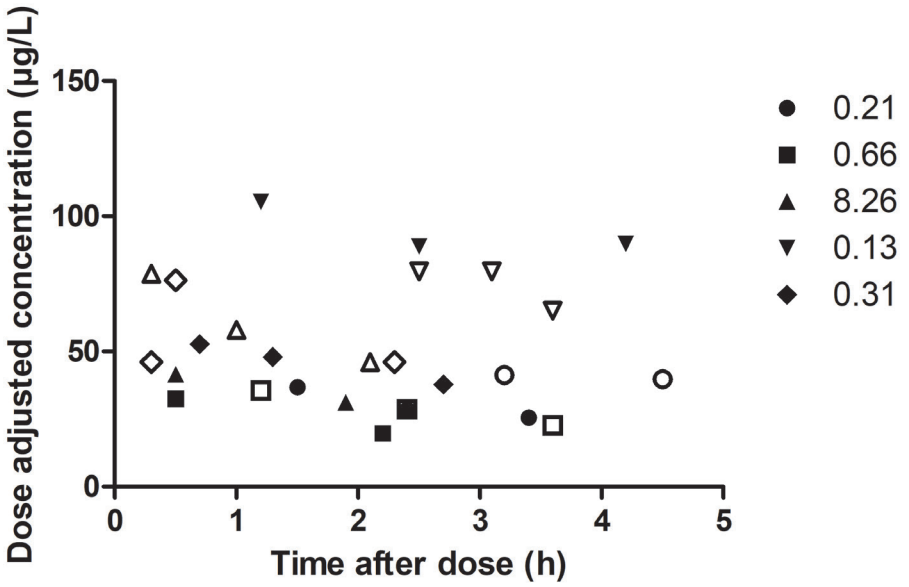

Figure 10.2 Dose adjusted plasma concentrations (0.05 mg/kg) after intravenous administration (solid symbols) and oral administration (open symbols) for individual patients receiving both intravenous and oral lorazepam. The legend displays the age in years.

Population pharmacokinetic model

The final dataset used in the NONMEM analysis consisted of 41 plasma concentrations from eight patients. The pharmacokinetic data were best described using a one-compartment model. Because of the low number of samples during the absorption phase, the estimates for ka after intravenous (immediate, 100 h^{-1}) and oral administration (fast, 10 h^{-1}) were both fixed. The population estimate, normalized for a body weight of 70 kg, was 82.6 L for Vd (relative standard error [RSE] 15 %), was 7.43 L h^{-1} for CL (RSE 14 %), and was 80.0 % for F (RSE 10 %). Shrinkage values were all below 10%. Estimating the exponent for allometric scaling did not result in a significant decrease in objective function value, so the exponent was fixed at 0.75 for CL and 1 for Vd. The covariate analysis did not result in significant covariates. The final model estimates are displayed in Table 10.2.

Table 10.2 Parameter estimates of final model and bootstrap analysis.

Parameter[a]	Final Model		Bootstrap (n=1000)		
	Point estimate	RSE (%)	2.5%	Median	97.5%
Vd (L)	82.6	15%	65.81	82.62	222.43
CL (L/h)	7.43	14%	5.48	7.22	9.60
F	0.80	10%	0.59	0.80	0.96
Interpatient variability (CV%)					
ηCL	32.1%	20%	11.5%	28.9%	43.8%
Residual variability	0.282	23%	0.111	0.258	0.373

[a] Vd (L) = 82.6*(WT/70) CL (L/h) = 7.43*(WT/70)0.75
CL clearance; Vd volume of distribution; F bioavailability; CV coefficient of variation ; RSE residual standard error

The goodness of fit plots in Figure 10.3 show that the population and individual predictions were evenly distributed around the line of identity when plotted against the observations. The bootstrap analysis of the final model showed that median parameter estimates were within 3% of population estimates from the original data set (Table 10.2). The visual predictive check indicated that the model adequately described the data.

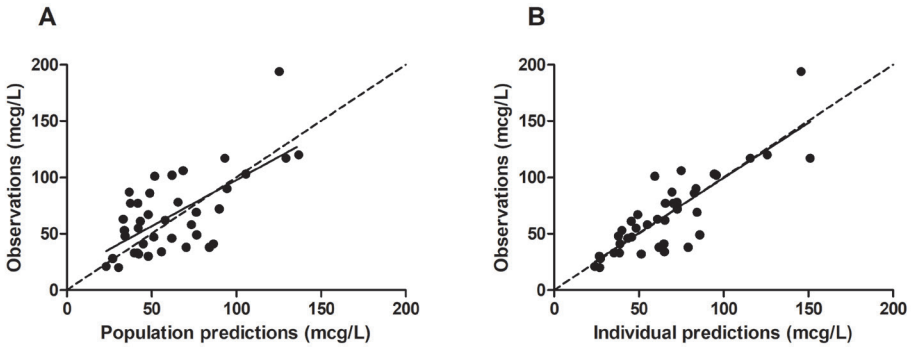

Figure 10.3 Goodness of fit plots of the final model. Population predictions vs. observations of lorazepam plasma concentrations (A) and individual predictions vs. observations of lorazepam plasma concentrations (B) The intermittent lines represent the line of identity, the solid lines represent the linear regression line.

Secondary endpoints

A limited number of sedation and withdrawal scores were available from the clinical nurse observations. During the pharmacokinetic sampling period, SOS scores were available for one patient, with scores ranging from 0-3, below the cut-off value for iatrogenic withdrawal syndrome. COMFORT-B scores were available for four patients, but only one of them had corresponding NISS scores available. These four patients all experienced minor incidents of oversedation, with incidental COMFORT-B scores of nine and ten. Three patients experienced a serious adverse event whilst still on study medication, but after the PK sampling period. One cardiac patient was reintubated as a result of a pulmonary *Pseudomonas aeruginosa* infection. Another cardiac patient experienced focal epilepsy, most likely caused by a subdural hematoma, and was treated with continuous intravenous midazolam. The last patient suffered from respiratory decline as a result of pre-existent pulmonary hypertension and was reintubated. All three incidents were considered to be unrelated to the study medication.

DISCUSSION

This study was the first to evaluate the pharmacokinetics and oral bioavailability of a lorazepam oral liquid in pediatric intensive care patients, using a population-based approach. The model constructed from the NONMEM analysis was able to predict the observed data with good precision.

The main pharmacokinetic parameter of interest, mean bioavailability after oral administration, was estimated at 80%, which is 10-15% lower than values previously reported for adults (11, 15). This difference was however not statistically significant, as the 95% confidence interval included the values reported for adults. In the case that bioavailability was indeed slightly lower in our patients, the most likely explanation is less precise dosing or a loss of drug during administration, rather than a reduced absorption or increased first pass-effect. Studies have shown that lorazepam exhibits sorption to PVC containing tubing (16), and due to fluid restrictions, rinsing of the tubing after drug administration was usually limited to a few milliliters of water, medication, nutrition, or even air. Further inaccuracies could have originated from dose volumes as low as 0.18 ml, which are difficult to measure accurately using the smallest available oral syringe of 1 ml.

Contrary to the previously reported pediatric population pharmacokinetic model by Gonzalez *et al* (5), our NONMEM analysis yielded no statistically significant covariates. With the small amount of pharmacokinetic samples and skewed distribution of age, body weight and sex among the study population, this was an expected result. Our data were also best described using a one-compartment model, whereas others report a two-compartment model to be the most suitable to describe the pharmacokinetics of lorazepam in both children and adults (5, 17). This finding can also be contributed to sparse sampling, but another explanation is that the oral absorption phase conceals the rapid distribution into the peripheral compartment. The major consequence of oversimplification of the distribution phase by using a one-compartment model would be that clearance and the terminal elimination rate could be overestimated. Indeed, our average clearance was higher and its derivative beta half-life was lower compared to the results of Gonzalez *et al* (5), but our population was much younger (median age 0.49 versus 5.4 years) and more severely ill. Normalized for a weight of 70 kg, our estimated clearance was 2.3 times higher compared to the referenced authors' model. Combined with our estimate for Vd, this leads to a 1,7 times lower estimate of elimination half-life of 11.1 hours. Although the average parameter estimates derived from our model fell well within the range reported in this study, it is possible that CL was overestimated. Nevertheless, due to the age distribution, weight-adjusted clearance could have been higher in our population.

In the prevention of IWS, gradual tapering of sedation is essential, since abrupt cessation or rapid weaning has been shown to precipitate withdrawal symptoms (18). For a patient of 5 kg, the estimated elimination half-life of 5.7 hours implies that a four times daily dosing schedule is not only required to prevent high peak concentrations, but also to maintain adequate lorazepam exposure between administrations. The pharmacokinetic parameters derived from our model suggest that even shorter dosing intervals could be necessary in the youngest patients to maintain adequate plasma concentrations. Adequate monitoring of symptoms of IWS remains therefore necessary.

A formal patient acceptability assessment of the lorazepam oral liquid could not be performed in the study population, as the product characteristics of palatability, swallowability and appearance were not applicable due to the mode of administration.

However, the liquid appears to be very suitable for its intended application in the pediatric ICU, with the exception of the administration of very low doses (<0.2 ml), which are difficult to measure accurately.

Some limitations of our study should be acknowledged. First, due to the minimally invasive design of the study, using a sparse pharmacokinetic sampling scheme, we did not collect sufficient samples to construct a two-compartment model or to fully explore potential significant covariates. Second, we did not determine lorazepam metabolite pharmacokinetics, which could have been used to improve the precision of CL estimates. Third, the initiation of lorazepam therapy was often a result of clinical improvement of the patient, which resulted in removal of the arterial line, and prevented the collection of enough pharmacokinetic samples. Fourth, for the collection of IWS and sedation scores, we relied on compliance to standard care protocols, but as with all clinical protocols, compliance is lower than expected, especially in this case when patients seemed adequately sedated. Despite these limitations, to the best of our knowledge, this is the first study to evaluate the pharmacokinetics of oral lorazepam in pediatric patients. For the majority of our patients we were able to collect pharmacokinetic samples after both intravenous and oral administration, which enabled us to demonstrate high oral bioavailability.

CONCLUSION

The current study provides important information regarding the pharmacokinetics of lorazepam after oral administration of the studied pediatric oral liquid formulation, and confirms its suitability for application in PICU patients. The estimated oral bioavailability of 80% does not support a correction factor when switching from intravenous to oral lorazepam. Our estimate for clearance in combination with previously reported values, indicate that a four times daily dosing schedule is necessary to maintain stable plasma levels of lorazepam, which are required to prevent iatrogenic withdrawal syndrome.

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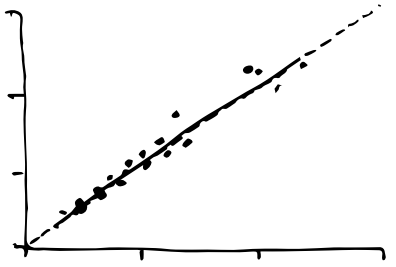

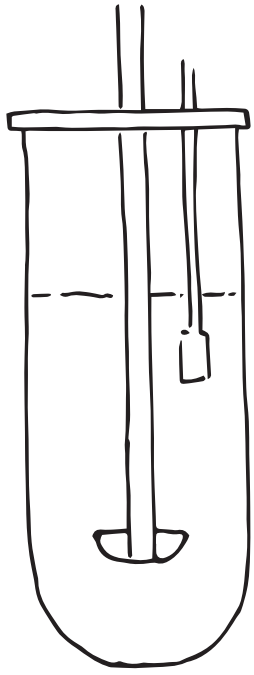

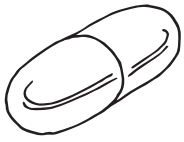

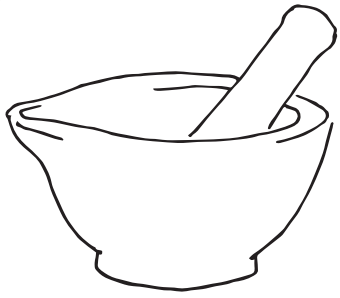

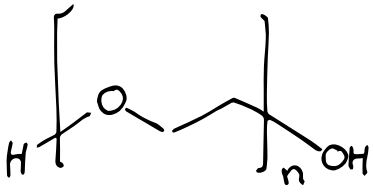
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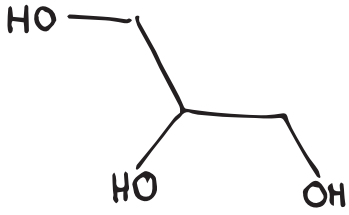
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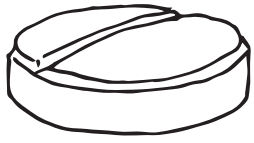

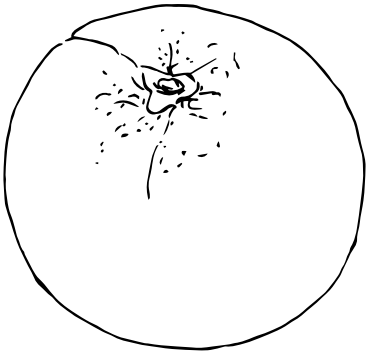

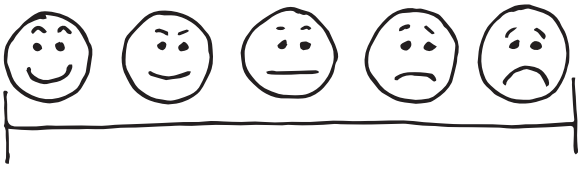

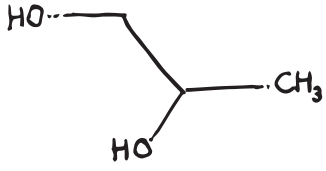
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Summarizing discussion

Children deserve access to medicines that have been specifically developed and researched for use in young patients. The measures that were put in place with the Paediatric Regulation have ensured that paediatric medicine development became an integral part of the overall development of medicines. However, for off-patent medicines, these measures have not been sufficient.

The research combined in this thesis aimes to improve paediatric pharmacotherapy by developing a standardised approach for the design and evaluation of pharmacy-compounded oral liquids of off-patents medicines. A multidisciplinary approach was sought with the intention to establish a framework for current and future paediatric formulation development, combining the expertise of pharmacists of the Laboratory of Dutch Pharmacists (LNA) and the Erasmus MC hospital pharmacy, and of clinicians of the Sophia Children's Hospital. Part 1 of this thesis consisted of a general introduction to the topic and an exploration of unmet needs and common practices relating to paediatric formulations in clinical practice. In part 2 we described the formulation development of two compounds chosen to represent both water-soluble and water-insoluble drugs, and for which an unmet need existed in paediatric practice. We presented *in vitro* methods to simulate *in vivo* performance of the developed liquids. In part 3 the results of the clinical studies were presented, in which the developed liquids were evaluated in both adult volunteers as well as the paediatric target population.

MAIN FINDINGS

Part 1: A large gap still exists between paediatric needs and the availability of medicines with an age-appropriate formulation. Pharmacy-compounded, unlicensed formulations remain essential to fulfil these needs. Manipulation of oral dosage forms is common practice and there is a need for improvement of information provision regarding manipulation towards parents/caregivers.

Part 1 of this thesis was funded by the Royal Dutch Pharmacists Association as part of the research programme of 2017. We identified the issues surrounding paediatric formulations in daily clinical practice, with the aim to guide future paediatric formulation development, and improve current information provision to parents and healthcare professionals regarding paediatric drug administration. Based on the dispensing data of the Sophia Children's Hospital, we identified a profound gap in the availability of age-appropriate formulations, especially for neonates and infants at the intensive care, for which 42% of the dispensed products were considered unsuitable, according to the acceptability matrix from the 'Reflection paper on formulations of choice for the paediatric population' (1). Our data show that pharmacy compounding in the treatment of paediatric patients remains essential, as more than half of the dispensed products did not have a marketing authorisation. A survey across Dutch paediatric hospital pharmacies revealed that the use of pharmacy-compounded products was widespread, and that almost half of the most commonly used compounded products in the Netherlands were not included in the EMA inventory of paediatric needs.

As part of the suitability assessment, exposure to potential toxic excipients was calculated based upon dosage and excipients concentration and compared with EMA limits for safe exposure. We found that possible toxic exposure was not limited to only neonatal ICU patients, but was relevant in children up to the age of four years. Efforts should be made to reduce the exposure to potentially harmful excipients, by avoiding or substituting non-

essential medicines, and improving the composition of essential medicines.

In chapter 3 we identified the problems in drug administrations to children, experienced by both parents/caregivers, as well as by nurses, by determining the extent, reasons and methods used for drug manipulation. The gap in availability of age-appropriate formulations was reflected in the results from this chapter. Manipulation of oral dosage forms was common practice among both parents/caregivers as well as nurses in a paediatric hospital, with a similar prevalence of 30% in the outpatient setting versus 37% in the inpatient setting. Manipulation by parents/caregivers occurred mainly to achieve taste and dose adjustment, whilst nurses most often used manipulation for administration through a feeding tube and size reduction. This difference probably results from the more extensive formulary of the inpatient pharmacy, which allows for more precise dosing with compounded liquids and capsules of different strengths, and the higher prevalence of feeding tubes in the inpatient setting.

The most unexpected result from the survey described in chapter 3 was the low dissemination of information regarding the correct method of manipulation from the pharmacy towards both parents/caregivers and nurses. Even though this information is available to pharmacies in the Dutch reference work Oralia VTGM, and within the hospital to the nurses through every workstation, only half of the interviewed parents/caregivers stated to have received their information from the pharmacy, and only 28% of the nurses consulted the pharmacy-provided information. Aside from this finding, many of the recommendations in the Oralia VTGM are based on practical experience, rather than research.

Part 2 The concept to develop two types of formulations, for water-soluble and water-insoluble drug compounds, appeared fertile for improving the availability of age-appropriate, paediatric formulations for off-patent drugs. Amlodipine and lorazepam can be compounded into stable oral clear solutions using simple techniques and safe excipients.

The second part of this thesis presented results from the ZonMw project that aimed to integrate pharmaceutical development of paediatric formulations and the consecutive clinical testing in the target population. Because of the need for flexible dosing and ease of administration, oral liquids were the preferred dosage form to be developed, and amlodipine and lorazepam were chosen to serve as proof of concept, and because of the unmet need in paediatric practice. As evidenced by chapter four and five of this thesis, the close cooperation with the LNA resulted in two feasible new formulations, both using safe and readily available excipients, requiring simple compounding techniques, and providing good stability when stored refrigerated.

Next to the pharmaceutical development , we explored the use of biopharmaceutical methods to predict *in vivo* performance of medicines in paediatric populations, facilitated by the University of Bath. With experiments designed to reflect clinical practice in the Sophia Children's Hospital, the impact of patient related factors on drug performance was studied, using drug solubility in paediatric biorelevant media and biorelevant dissolution. Ideally, these *in vitro* predictive methods, combined with *in silico* models, will in the future replace *in vivo* experiments and clinical trials in paediatric patients.

Part 3 The theoretical approach from part two resulted in clinically useful formulations. The amlodipine oral solution is bioequivalent to amlodipine tablets, and both the amlodipine and lorazepam oral solutions provide high oral bioavailability

The third part of this thesis consists largely of clinical research. As part of the ZonMw project, both formulations were further studied in the target population to assess pharmacokinetic parameters, safety issues and acceptability. For amlodipine, we chose to first compare the performance of the oral solution to originator tablets in adult volunteers in a bioequivalence study. As expected, the oral solution and tablets were bioequivalent, with only a statistically different time to maximum concentration. With the slow and passive absorption of amlodipine, this difference is expected to have no clinical relevant effect on blood pressure control. The consecutive population pharmacokinetic study in paediatric patients confirmed the oral solution to be a good treatment option for younger paediatric patients with adequate acceptability. The population pharmacokinetic study of the lorazepam oral solution in paediatric intensive care patients was the first study to evaluate oral lorazepam in paediatric patients. Using a population pharmacokinetic approach and non-linear mixed effects modelling, we demonstrated high oral bioavailability of 80% for the lorazepam oral solution.

METHODOLOGICAL CONSIDERATIONS

Strengths and limitations

The major strengths of the studies included in this thesis relate to

- the large datasets collected at the Sophia Children's Hospital, representing the entire paediatric age range and all major and minor specialties
- the multidisciplinary approach, combining the expertise of pharmacist and paediatricians, and based on clinical practice of the largest paediatric hospital of The Netherlands
- the conformity of the results of the clinical trials with our expectations and available literature

One of the main strengths of this thesis generates from the multidisciplinary approach, which ultimately resulted in the development of two paediatric oral solutions, which are supported by clinical data from the target population, and can be considered standard of care following incorporation into the Formulary of Dutch Pharmacists (FNA). The composition, method of preparation and shelf-life make both oral solutions suitable for large-scale production as well as extemporaneous compounding. The formulation design and validation was supported by the experts of the LNA, and the Department of Pharmaceutical Technology and Biopharmacy of the University of Groningen. The collaboration with the University of Bath showed that *in vitro* biopharmaceutical tools can be useful for studying drug performance in children. The straightforward experimental setups make it possible to address numerous different administration scenarios, which would not be feasible or ethical in pharmacokinetic studies in children.

The clinical phase of the ZonMw project was designed to perform patient-based research in the target paediatric population, aiming to elucidate pharmacokinetic, acceptability and safety parameters of the developed oral solutions. Both paediatric trials were designed in close collaboration with the clinicians, and the lorazepam trial profited from the well-established clinical research structure of the paediatric intensive care unit. We were able to include patients in a difficult setting, and as young as 4 weeks old. Furthermore, the clinical trial results were in accordance with our expectations based on the physical-chemical characteristics of the compounds and previously reported studies in both adults and children.

The most important general limitations of the studies included in this thesis relate to

- gaps in the knowledge base regarding acceptability of medicines to paediatric populations
- a knowledge gap concerning gastro-intestinal physiology in paediatric patients, limiting the predictive value of the biopharmaceutical *in vitro* experiments
- due to refusal of the parents (amlodipine) and absence of an arterial line (lorazepam), inclusion rates in the paediatric trials were low

Guidance issued by the European Medicines Agency states that patient acceptability must be an integral part of paediatric formulation development and be described in the paediatric investigation plan (PIP) (2), but before this guidance came into effect in 2014, there was no requirement for medicines to be demonstrated to be acceptable to children. The evidence base concerning what is acceptable to paediatric patients is therefore limited and standard methods or criteria that define what is considered acceptable have not been determined (3). The suitability assessment in chapter 2 is based on the acceptability matrix from the 'Reflection paper on formulations of choice for the paediatric population' by the EMA (1), but the matrix was based on expert opinion rather than sound scientific evidence, which limits the validity of the results.

The solubility and dissolution experiments presented in chapter 6 explore biopharmaceutical tools that can be used to predict *in vivo* drug performance. Ideally, the results obtained from *in vitro* dissolution experiments would be integrated into more complex *in silico* prediction models. This physiologically based pharmacokinetic (PBPK) modelling and simulation is already commonly used in formulation development/bridging for adult medicines and provides a promising tool for paediatric *in vivo* drug performance prediction, provided we gain a better understanding of the developmental changes of the gastrointestinal tract in the paediatric population (4). Furthermore, validation of the biopharmaceutical methods requires rich PK data, which are often not available.

In the lorazepam trial, removal of the arterial line to prevent infection and/or discharge to the general ward often resulted in eligible patients not participating in the study. For the amlodipine trial, refusal by the parents due to the burden of study procedures was common. This led to lower than expected inclusion rates, which is commonly referred to as Lasagna's Law, where "the incidence of patient availability sharply decreases when a clinical trial begins and returns to its original level as soon as the trial is completed" (5).

Study endpoints and feasible trial design

Initially, the amlodipine paediatric trial was designed to compare formulation performance of tablets and our oral solution. From *in vitro* studies and adult data we already knew that the oral pharmacokinetics of amlodipine are minimally influenced by the dosage form (6), which was confirmed in our bioequivalence study in healthy adults. Also, ICH E11 clearly states that relative bioavailability comparisons of paediatric formulations with the adult oral formulation should be done in adults, unless the drug is unsafe in healthy volunteers, the PK of the compound is different in patients, or the PK of the compound is different in children (7). Since amlodipine is absorbed by slow passive diffusion across the intestinal membrane, differences in intestinal drug absorption between adults and paediatric patients are unlikely. A comparison of formulation performance in paediatric patients was therefore in hindsight not indicated. With an amendment, we changed the focus of the trial to elucidation of the pharmacokinetic parameters of amlodipine in children, with secondary endpoints regarding acceptability, pharmacodynamics (blood pressure) and clinical covariates, but ultimately only acceptability was a formulation specific outcome. During the conduct of the study, it became clear that the study procedures and switching to study medication were considered a burden to many of the eligible patients and parents, and were reasons not to participate in the trial. Consequently, inclusion of study participants did not reach the goal of 20 patients.

Pharmacokinetic data of amlodipine in children under the age of six years are still warranted, but are formulation independent, which we were not aware of at the start of the project. This provides the opportunity to collect them in less invasive manner, for instance, from renal transplant patients that regularly undergo blood sampling for therapeutic drug monitoring of immunosuppressants. To collect pharmacokinetic data from the youngest patients, study procedures could be limited to collection of capillary blood samples, which is for many patients a regular procedure with a low burden.

The lorazepam trial was well designed for its purpose of determining oral bioavailability, which we accomplished with inclusion of only eight patients. Even though there were no indications that lorazepam would perform different in paediatric patients compared to adults, we have now confirmed this in a relatively non-invasive trial in the relevant population. From personal experience, inclusion rates could have been improved with a slightly different approach, which was implemented with the second study amendment. Introducing the study to the parents became easier when the lorazepam oral solution became standard of care and replaced the previously compounded 0.1 mg capsules. Initially, the study was designed to include only patients who were yet to start with lorazepam, but this was actually no requirement for the determination of oral bioavailability when using non-linear mixed effects modelling. The single administration of an intravenous dose was no objection for any of the parents. Unfortunately, the presence of an arterial line proved to be essential for the successful collection of blood samples, and was a factor we could not influence. It shows how complicated clinical research in paediatric patients can be. The acceptability of the oral solution could not be assessed in this population, as all patients received it through a feeding tube. It is expected that the formulation will incidentally be applied in the outpatients setting, where this formulation property will become more relevant.

RECOMMENDATIONS

Recommendations for practice

Even though no immediate risks were identified in the survey regarding manipulation, pharmacist should improve their efforts in proactively informing parents/caregivers about drug manipulation and administration, and this should include both verbal as well as written information. The Royal Dutch Pharmacists Association could support this effort with the development of patient-oriented, generic information leaflets regarding manipulation techniques, most importantly dose adjustment of solid dosage forms.

Recommendations for policy

As shown in the amlodipine trial, the availability a suitable formulation can greatly improve the ease of drug administration to children, and subsequently, have an influence on treatment outcome. It is essential that pharmacists keep investing in the development of suitable formulations for paediatric patients, in collaboration with paediatricians, the LNA and compounding pharmacies within The Netherlands. The special interest group 'paediatrics' of the Dutch Association of Hospital Pharmacists should take the lead in this.

The efforts of the European Pharmacopoeia Commission in the compilation of a pan-European Paediatric Formulary should be highly supported. Information collected in the Formulary of Dutch Pharmacists could be valuable. Further financial support from the European Union could accelerate the efforts and is necessary for standardisation, validation and filling the gaps in information, and would, in our opinion, be well-spent.

Standard methods or criteria that define what is considered acceptable to children have not been determined (3). The approach that was chosen in chapter eight to study the acceptability of the amlodipine oral liquid is generally considered suitable, but the lack of standardisation makes comparing results difficult. A lack of knowledge about what is currently considered to be acceptable to paediatric patients hinders the development of acceptable, age-appropriate medicines. Therefore, EMA guidance on how to perform and interpret acceptability studies in paediatric patients is highly warranted.

Recommendations for future research

The oral solutions presented in part 2 of this thesis were meant to serve as proof of concept, and the drug substances were chosen to represent water-soluble and water-insoluble compounds. The approach that was chosen to process the poorly water-soluble lorazepam, using a mixture of organic solvents, should be tested for other drug substances with poor aqueous solubility. The readily available and cheap excipients, and the relatively easy compounding method, could possibly provide a solution for a large range of difficult to process drug substances. Compound selection should focus on BCS class II and class IV drugs.

The paediatric population remains a difficult population to study. Clinical trials are expensive, and resources should be allocated wisely. Many trials fail or are not completed, and the reasons for that are several (8). It is very likely that paediatric drug development will benefit from European collaboration, as envisioned by the Connect4Children collaborative network for European clinical trials for children, which aims to generate a

sustainable infrastructure that optimises the delivery of clinical trials in children.

In vitro biopharmaceutical techniques, combined with *in silico* models, have the potential to replace *in vivo* experiments and clinical trials, but there is still a knowledge gap concerning GI physiology in paediatric patients. Aside from the factors influencing in vivo dissolution, specific research is still required on the factors influencing permeability, mainly the ontogeny of metabolizing enzymes and drug transporters, to better predict oral drug absorption in this population. Access to existing paediatric rich pharmacokinetic data is required to validate the biopharmaceutical tools.

In this thesis we have shown that for off-patent medicines, for which there is no economics basis for licensing, pharmacy compounding may offer a highly feasible solution to provide acceptable and dose flexible pharmacotherapy for children.

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Samenvatting

Geneesmiddelonderzoek bij kinderen heeft lange tijd te weinig aandacht gehad. Tot ver in de 20e eeuw was men van mening dat kinderen niet zouden mogen deelnemen aan klinisch geneesmiddelonderzoek, met name vanwege ethische bezwaren. Tegenwoordig is de algemene consensus dat kinderen recht hebben op toegang tot geneesmiddelen die specifiek voor hen ontwikkeld en onderzocht zijn. Dat neemt niet weg dat er nog talloze obstakels te overbruggen zijn. Met name de heterogeniteit binnen de vaak toch al kleine studiepopulatie, maakt het opzetten van goede kindergeneesmiddelonderzoeken een uitdaging. Daarnaast maken de veelal kleine doelpopulaties het economisch onaantrekkelijk voor bedrijven om te investeren in geneesmiddelregistraties specifiek voor kinderen.

Om dit probleem aan te pakken werd ,in navolging van de Verenigde Staten, in 2006 in de Europese Unie de Paediatric Regulation aangenomen, wat ertoe heeft geleid dat in de jaren 2007-2016 meer dan 260 nieuwe geneesmiddelen en indicaties voor gebruik door kinderen zijn goedgekeurd door de European Medicines Agency (EMA). Farmaceutische bedrijven beschouwen de ontwikkeling van kindergeneesmiddelen nu als integraal onderdeel van het ontwikkeltraject van een geneesmiddel. Hier tegenover staat dat de ontwikkeling van middelen die uit patent zijn is achtergebleven.

Er zijn veel 'oudere' geneesmiddelen die een belangrijke plaats hebben in de behandeling van kinderen, maar vaak is hiervan geen geschikte toedieningsvorm beschikbaar. Met name de acceptatie door de patiënt (o.a. op basis van smaak) en de dosisflexibiliteit vormen vaak een probleem. Apothekers kunnen in een dergelijk geval zelf een geneesmiddel bereiden, zogenaamde magistrale bereidingen. Dit heeft vaak de voorkeur boven het manipuleren van bestaande toedieningsvormen, zoals het vermalen van tabletten, of toediening met dranken of voeding. Magistrale bereidingen worden in Nederland meestal volgens standaardvoorschriften gemaakt (Formularium der Nederlandse Apothekers (FNA)) hoewel dat niet verplicht is. FNA-voorschriften worden farmaceutisch-technisch uitgebreid onderzocht en wanneer deze onder de juiste omstandigheden worden bereid kan de kwaliteit gegarandeerd worden. Buiten het FNA worden er echter nog talloze niet-gestandaarde bereidingen toegepast die qua samenstelling sterk kunnen verschillen tussen de verschillende kinderziekenhuizen. Het is de vraag of het ontwerp van deze producten optimaal is voor toepassing bij kinderen.

Deel 1 Kinderformuleringen in de dagelijkse klinische praktijk

In hoofdstuk 2 van dit proefschrift hebben we in kaart gebracht welke plaats de apotheekbereiding inneemt in de behandeling van klinische patiënten van het Erasmus MC Sophia Kinderziekenhuis. Met name neonaten, zowel prematuur als aterm geboren, werden vaak behandeld met eigen bereidingen, die meer dan de helft van de afgeleverde geneesmiddelen vormden. Ook werd duidelijk dat er in Nederland veel eigen bereidingen worden toegepast die niet in de EMA inventory of paediatric needs zijn opgenomen, terwijl deze middelen dus kennelijk wel nodig zijn. Naast de focus op apotheekbereidingen hebben we in dit hoofdstuk ook onderzocht hoe groot de blootstelling aan potentieel schadelijke hulpstoffen was bij klinische patiënten, met een focus op vloeibare geneesmiddelen. Hieruit bleek dat er verbeteringen te behalen vielen door middel van substitutie van bepaalde producten en het verbeteren van de samenstelling van bepaalde eigen bereidingen.

Als er geen goede toedieningsvorm beschikbaar is, wordt vaak teruggevallen op

manipulatie van de toedieningsvorm door ouders en/of zorgverleners, bijvoorbeeld door tabletten te vermalen, ze op te oplossen, te breken of ze vermengd met melk of eten toe te dienen. De consequenties van het manipuleren op de effectiviteit en veiligheid van het geneesmiddel zijn niet duidelijk of soms zelfs bewezen schadelijk. Uit het onderzoek beschreven in hoofdstuk 3 onder poliklinische patiënten in het Erasmus MC Sophia Kinderziekenhuis bleek 45% van de ondervraagde ouders orale medicatie te manipuleren voor toediening. In de praktijk zijn instructies aan ouders over manipulatie-mogelijkheden vaak beperkt, niet uniform en veelal niet goed onderbouwd. Eén van de aanbevelingen die uit dit proefschrift volgen is dan ook om deze informatievoorziening door apothekers te verbeteren, en waar nodig deze informatie ook te genereren.

Deel 2 Farmaceutische ontwikkeling en *in vitro* evaluatie

Er is een grote behoefte aan goed onderzochte, kindvriendelijke, orale geneesmiddelen, die bij voorkeur een grote dosisflexibiliteit hebben. In het kader van het programma Priority Medicines voor Kinderen heeft ZonMW hiervoor een subsidie verstrekt, waarmee de ontwikkeling van twee dranken is bekostigd. Uitgangspunt was hierbij dat de formuleringen toepasbaar zouden zijn voor meerdere geneesmiddelen. Amlodipine en lorazepam zijn vervolgens gekozen als modelstoffen voor water-oplosbare en niet water-oplosbare geneesmiddelen.

In samenwerking met het Laboratorium der Nederlandse Apothekers (LNA) werd gestart met de farmaceutische ontwikkeling van twee dranken, rekening houdend met de beperkte hoeveelheid hulpstoffen die veilig gebruikt kunnen worden en specifieke aspecten zoals smaak (acceptatie). Om doseerfouten van potente middelen te voorkomen gaat de voorkeur uit naar een heldere drank boven een suspensie, omdat bij een suspensie omschudden nodig is voor dosis homogeniteit. In de praktijk zijn ernstige fouten voorgekomen bij toepassing van inhomogene suspensies. Op basis van de fysisch-chemische eigenschappen (oplosbaarheid, pKa) van het geneesmiddel is gekeken welke oplosvloeistoffen mogelijk waren, welke pH nagestreefd moest worden en welke hulpstoffen daarbij noodzakelijk waren. Vervolgens is houdbaarheidsonderzoek uitgevoerd met gevalideerde analysemethodes. Dit heeft uiteindelijk geresulteerd in de ontwikkeling van een amlodipinedrank van 0,5 mg/ml (hoofdstuk 4) en een lorazepamdrank van 1 mg/ml (hoofdstuk 5).

Naast de ontwikkeling van de twee dranken is in samenwerking met de Universiteit van Bath onderzoek gedaan naar *in vitro* modellen die de blootstelling aan orale geneesmiddelen bij kinderen kunnen voorspellen. Hierbij is de vrijgifte van twee geneesmiddelen onderzocht in nagebootste vloeistoffen uit het maagdarmkanaal. Hiermee kan een voorspelling worden gedaan over de uiteindelijke blootstelling bij toediening aan patiënten. Het is de bedoeling dat, in de toekomst, deze modellen het *in vivo* onderzoek bij kinderen grotendeels overbodig maken.

Deel 3 Klinische toepassing van de formuleringen

Om de blootstelling aan twee verschillende varianten van hetzelfde geneesmiddel te vergelijken wordt bio-equivalentieonderzoek bij volwassen vrijwilligers uitgevoerd. De farmacokinetische parameters area under the curve en de maximale plasmaconcentratie na een eenmalige dosis van het onderzoeksmiddel en een referentiemiddel worden vergeleken, als het verschil binnen bepaalde grenzen valt worden de middelen

beschouwd als bio-equivalent. Uit de bio-equivalentiestudie met amlodipine beschreven in hoofdstuk 7 bleek dat de drank en tabletten gelijkwaardig waren. Voor lorazepam is geen bio-equivalentieonderzoek uitgevoerd, omdat er geen relevant, bij kinderen toegepast product was om mee te vergelijken. Gezien de fysisch-chemische eigenschappen van lorazepam is er ook geen groot verschil te verwachten tussen verschillende producten.

De volgende fase was de toepassing van de dranken bij kinderen, waarbij farmacokinetiek (PK), farmacodynamiek (PD), bijwerkingen en de acceptatie in kaart gebracht werden. Voor amlodipine werd onderzoek uitgevoerd bij patiënten (6 maanden-11 jaar) met hypertensie, voor lorazepam bij kinder-IC-patiënten (0-11 jaar). Alle geïncludeerde patiënten gebruikten het geneesmiddel om klinische redenen.

Met software om patiëntendata te modelleren (Non-lineair Mixed Effects Modeling, NONMEM®) was het mogelijk om ook met beperkte datasets en wisselende bloedafnametijdstippen resultaten te genereren. Deze lieten zien dat beide dranken voorzagen in adequate bloedspiegels, er werden geen ernstige bijwerkingen waargenomen gerelateerd aan de dranken en ze werden goed geaccepteerd door de doelgroep. Bij de lorazepamstudie was de doelgroep een kwetsbare, instabiele groep op de IC met veel co-morbiditeit en co-medicatie, maar ouders bleken toch open te staan voor deelname van hun kind aan onderzoek.

De laatste jaren wordt het belang van goede toedieningsvormen van geneesmiddelen voor kinderen steeds meer erkend. Apotheekbereidingen spelen hierbij een belangrijke rol vanwege het ontbreken van handelsproducten. Optimalisatie en standaardisatie van deze bereidingen is noodzakelijk uit oogpunt van kwaliteit. Bij de ontwikkeling moet aandacht zijn voor dosisflexibiliteit en de geschiktheid voor neonaten en jonge kinderen, met name ten aanzien van hulpstoffen. Een samenwerking tussen kinderartsen en apothekers is hierbij belangrijk om de behoefte in de klinische praktijk adequaat te kunnen invullen. In dit onderzoek heeft dat geleid tot de succesvolle ontwikkeling en toepassing van amlodipine- en lorazepamdrank bij kinderen.

Summary

Drug development for children has long been a neglected area compared to adult drug development. Until late into the 20th century, the general view was that children should not participate in clinical trials, particularly because of ethical concerns. Today, the general consensus is that children are entitled to medicines that have been specifically developed and researched for them. Nevertheless, many barriers still remain. In particular, the heterogeneity within the already very small study population makes setting up good paediatric drug researches a challenge. In addition, the mostly small target populations make it economically unattractive for companies to invest in drug registrations specifically for children. To address these issues, the Paediatric Regulation was adopted in the European Union in 2006, leading to more than 260 new medicines and indications for use by children approved by the European Medicines Agency (EMA) in 2007-2016. Pharmaceutical companies now consider the development of paediatric medicines as an integral part of the development process of a medicine. On the other hand, the development of off-patent medicines lags behind.

There are many 'older' medicines that have an important place in the treatment of children, but often no suitable dosage form is available. In particular, acceptance by the patient (e.g. based on taste) and dose flexibility are a problem. Pharmacists can in such a case compound a medicine, so-called *magistral* preparations. This is often preferred over manipulating existing dosage forms, such as grinding of tablets, or administration with drinks or food. In the Netherlands, m*agistral* preparations are usually made according to standard instructions (Formulary of Dutch Pharmacists (FNA)), although this is not mandatory. FNA products are extensively studied regarding pharmaceutical quality, and when they are prepared under the right conditions the quality can be assured. However, numerous non-standard preparations are still being used outside the FNA, which differ greatly in composition between the different children's hospitals. The question is whether the design of these products is optimal for application in children.

Part 1 Paediatric formulations in daily clinical practice

In chapter 2 of this thesis we demonstrated the importance of pharmacy preparation in the treatment of clinical patients at the Erasmus MC Sophia Children's Hospital. In particular, neonates, born prematurely and term, were often treated with pharmacy preparations, which accounted for more than half of the medicines dispensed. It also became clear that many pharmacy preparations used in the Netherlands are not included in the EMA inventory or paediatric needs, while these medicines are obviously needed. In addition to the focus on pharmacy preparations, in this chapter we also investigated the extent of exposure to potentially harmful excipients in clinical patients, with a focus on liquid medicines. This showed that improvements could be achieved by substituting certain products and improving the composition of certain pharmacy preparations.

If a suitable dosage form is not available, parents and/or caregivers often rely on manipulation of the dosage form, for example by grinding tablets, dissolving them, breaking them or mixing them with milk or food. The consequences of manipulating on the effectiveness and safety of the drug are not clear, or even proven to be harmful. From the research described in chapter 3 among outpatients at the Erasmus MC Sophia Children's Hospital, 45% of the parents participating in the questionnaire indicated to manipulate oral medication for administration. In practice, instructions to parents about manipulation options are often limited, not uniform and often not well substantiated. One of the recommendations that follows from this thesis is therefore to improve this

information provision by pharmacists and, where necessary, to generate this information.

Part 2 Pharmaceutical development and in vitro evaluation

There is a great need for well-studied, child-friendly, oral drugs, which preferably have a large dose flexibility. Under the ZonMW Priority Medicines program for children, ZonMW has provided a subsidy, which has funded the development of two liquid formulations. The starting point was that the composition of the formulations would be suitable for several drugs. Amlodipine and lorazepam were then chosen as model compounds for water-soluble and non-water-soluble drugs.

In collaboration with the Laboratory of Dutch Pharmacists (LNA), the pharmaceutical development of two liquid formulations was started, taking into account the limited amount of excipients that can be safely used, and specific aspects such as taste (acceptance). In order to prevent dosing errors of potent agents, preference is given to a clear liquid over a suspension, because in a suspension shaking is necessary for dose homogeneity. In practice, serious errors have occurred with the use of inhomogeneous suspensions. On the basis of the physicochemical properties (solubility, pKa) of the compounds, we examined which solvents were possible, which pH had to be sought and which excipients were necessary. Subsequently, stability testing was performed using validated analysis methods. This ultimately resulted in the development of an amlodipine oral solution of 0.5 mg/ml (chapter 4) and a lorazepam oral solutions of 1 mg/ml (chapter 5).

In addition to the development of the two liquids, in collaboration with the University of Bath research was done into *in vitro* models that can predict the exposure to oral medicines in children. Here, the release of two drugs was investigated in simulated fluids from the gastrointestinal tract. This allows a prediction to be made about the drug exposure when administered to patients. The intention is that, in the future, these models will largely replace *in vivo* research in children.

Part 3 Clinical application of the formulations

To compare the exposure to two different variants of the same drug, bioequivalence testing is performed in adult volunteers. The pharmacokinetic parameters area under the curve and the maximum plasma concentration after a single dose of the study drug and a reference product are compared, and if the difference falls within certain limits, the products are considered bioequivalent. The bioequivalence study with amlodipine described in chapter 7 showed that the oral solution and tablets were equivalent. For lorazepam, no bioequivalence study was performed, because there was no relevant product used in children to compare with. Given the physical-chemical properties of lorazepam, no major difference can be expected between different products.

The next phase was studying the formulations in paediatric patients, in which pharmacokinetics (PK), pharmacodynamics (PD), side effects and acceptance were investigated. For amlodipine, a study was conducted in patients (6 months -11 years) with hypertension, for lorazepam in paediatric intensive care patients (0-11 years). All included patients used the drug for clinical reasons.

With software to model patient data (Nonlinear Mixed Effects Modeling, NONMEM®), it was possible to generate results with limited data sets and changing blood sampling

times. These showed that both liquids provided adequate blood levels, no serious side effects were observed related to the study drug and the liquids were well accepted by the target group. In the lorazepam study, our patients were vulnerable, sometimes unstable ICU patients with a lot of co-morbidity and co-medication, but parents turned out to be open to participation of their child in research.

In recent years, the importance of suitable dosage forms for children has been increasingly recognized. Pharmacy preparations play an important role here because of the lack of commercial products. Optimization and standardization of these preparations is necessary to guarantee good quality. Dose flexibility and the suitability for neonates and young children, particularly with regard to excipients, should be considered in the development of formulations for children. A collaboration between paediatricians and pharmacists is important in order to adequately fill the need in clinical practice. This research has led to the successful development and application of amlodipine and lorazepam liquid formulations in children.

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About the author

LIST OF PUBLICATIONS

1. Van der Vossen AC, van Nuland M, Ista WG, de Wildt SN, Hanff LM. Oral lorazepam can be substituted for intravenous midazolam when weaning paediatric intensive care patients off sedation. Acta Paediatr. 2018 Mar 23 [Epub]

2. Van der Vossen AC, van der Velde I, Smeets OS, Postma DJ, Eckhardt M, Vermes A, Koch BC, Vulto AG, Hanff LM. Formulating a poorly water soluble drug into an oral solution suitable for paediatric patients; lorazepam as a model drug. Eur J Pharm Sci. 2017 Mar 30;100:205-210.

3. Van der Vossen AC, van der Velde I, Smeets OS, Postma DJ, Vermes A, Koch BC, Vulto AG, Hanff LM. Design and stability study of an oral solution of amlodipine besylate for pediatric patients. Eur J Pharm Sci. 2016 Sep 20;92:220-3.

4. Van der Vossen AC, van der Velde I, van den Meiracker AH, van der Nagel BC, Koch BC, Vulto AG, Hanff LM. Bioequivalence study of an extemporaneously prepared oral solution of amlodipine suitable for use in pediatric patients compared to commercial tablets. Int J Clin Pharmacol Ther. 2016 Jan;54(1):65-72.

5. Van der Vossen AC. Marketing Authorisations under Exceptional Circumstances for Oncology Drugs. Ludwig Boltzmann Gesellschaft GmbH. 2013 Feb http://eprints.hta.lbg.ac.at/992/#

PHD PORTFOLIO

Courses	Month/Year	Workload	ECTS
(UMCU) Basiscursus Regelgeving en Organisatie voor Klinisch onderzoekers	Oct 2014	32 hrs	
(NIHES) Biostatistics for Clinicians (EWP22)	Feb 2015	25 hrs	
(EUR) Biomedical English Writing and Communication	Mar-May 2015		3
(EUR) Research Integrity	Nov 2015		0,3
(EUR) Basisdidactiek voor docenten (TtT I)	Jan 2016	16 hrs	
Seminars and workshops			
(Medical Library) Endnote	Aug 2014	4 hrs	
(EUR) Open Clinica Training	Sept 2014	8 hrs	
(Medical Library) Systematic Literature Retrieval (Pubmed)	Nov 2014	8 hrs	
(Medical Library) Systematic Literature Retrieval (Other databases)	Jan 2015	4 hrs	
Care for Pharmacy	Mar 2015	12 hrs	
(LUMC) Interpreteren van PK/PD studies	Apr 2015	4 hrs	
TULIPS Young Researchers Day	Nov 2015	8 hrs	
NVT Spring Symposium 'Pediatric drug development: a field in maturation'	Mar 2016	4 hrs	
(EUR) Omgaan met groepen	Apr 2016	4 hrs	
(EUR) Basistraining Limesurvey	Jun 2016	3 hrs	
Teaching			
(EUR Medicine) Interacties, VO Receptschrijven, Antibiotica-profylaxe en Antistolling	2015-present	24 hrs/year	
(UU Pharmacy) Introduction to clinical research in children (2/year)	2016/2017	4 hrs/year	
Supervision Master Thesis			
Kadir Akçay	Aug 2016 - Feb 2017		2
Sid Makhan	Feb 2017 – Jul 2017		2
Sandra Buljaç	Sept 2017 - Mar 2018		2
Conferences			
7th EuPFI Conference Antwerp (Poster)	Sept 2015	32 hrs	
14th International Congress of TDM and Clinical Toxicology Rotterdam	Oct 2015	8 hrs	
8th EuPFI Conference Lisbon (Poster)	Sept 2016	32 hrs	
FIGON/DMD (Poster)	Oct 2016	24 hrs	
9th EuPFI Conference Warschau (Poster)	Sept 2017	32 hrs	
Nederlandse Ziekenhuisfarmacie dagen (Oral)	Nov 2017	24 hrs	

CURRICULUM VITAE

Annette van der Vossen was born on the 11th of May 1988 in Leidschendam, and grew up in Voorburg, The Netherlands. After graduating from secondary school at Huygens Lyceum in Voorburg in 2006, she started her Pharmacy studies at Utrecht University. She obtained her bachelor's degree in 2011 and her master's degree in 2014. During her master's studies, she spent six months in Vienna, Austria, to perform a research project at the Ludwig Boltzmann Institut für Health Technology Assessment.

Annette started her professional career at the Department of Pharmacy of the Erasmus MC, University Medical Center Rotterdam, were she performed the research presented in this thesis. She was supervised by Promotor prof. dr. A.G. Vulto, and co-promotor dr. L.M. Hanff. During the spring of 2017, she spent three months at the University of Bath, Department of Pharmacy and Pharmacology, for a research visit under supervision of dr. N. Fotaki.

As of July 2018 she is working a the hospital pharmacy of the Maasstadziekenhuis in Rotterdam.