MINIMALLY INVASIVE METHODOLOGY FOR PHARMACOLOGICAL RESEARCH INVOLVING CHILDREN

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CHAPTER 1

General Introduction

Background

Children represent a unique population in the context of pharmacotherapy. They differ from adults in drug disposition (pharmacokinetics) and drug response (pharmacodynamics). The indications for prescribing medication cover a different range of conditions when comparing to adults. As, until recently, the pharmaceutical industry was not required to submit data concerning children to the competent authorities when applying for a marketing authorization, many pharmaceutical compounds lack pediatric data in their registration files. Nevertheless, many of these compounds are frequently prescribed to children. Consequently, children are often prescribed drugs outside the registered indication, a phenomenon referred to as off-label prescribing: i.e. use of a licensed drug in a way differing from the labeled indication, age, dose or administration route (1). Besides off-label use, unlicensed drug use is also frequent in pediatrics: this is the case when licensed medication is modified prior to administration (for example tablets crushed and suspended) or chemicals are used as medicine. Depending on the setting and the population studied, up to 100% of prescriptions to children are off-label or unlicensed (2).

Where it might seem logical to extrapolate efficacy and safety data found in the adult population to the pediatric population, this approach might lead to unexpected adverse events caused by nonlinear development of physiology from childhood into adulthood, affecting both drug disposition and drug response. This of course may lead to unexpected (side-) effects.

Throughout history there are numerous infamous incidents illustrating how safety issues can emerge when drugs are not researched adequately before use in children. The antibiotic chloramphenicol was found to result in the lethal 'grey baby syndrome' caused by slow metabolization, and as a result accumulation of toxic levels of the drug in neonates (3). Thalidomide administered to pregnant women led to abnormal development of the limbs in the developing fetus (4). Not only the active compound may lead to safety issues: also the use of excipients can cause harm. For example, in the 1930's the antibiotic sulfanilamide was marketed as a liquid formulation suitable for administration to children. Diethylene glycol was used as dissolving agent. As establishing drug safety was not required prior to marketing, this led to the death of 107 persons including many children from diethylene glycol poisoning (5).

Increasing safety of drugs for children

In both the United States and in Europe, legislators have responded to the aforementioned disasters with legislation increasing their control over marketed compounds. Without going into detail, the focus of these new or amended laws surrounded establishment of both safety and efficacy of compounds prior to marketing. Ironically, although most major drug-related disasters incidents occurred in children, the majority of the laws and regulations benefitted mainly adult drug users. It was not until the 1990's that legislators started deploying powerful instruments ensuring that safety and efficacy of new compounds had also to be studied in the pediatric age groups, after they recognized the lack of available data concerning children and the large proportion of off-label and unlicensed use of medication in children.

In Europe, this legislation came into full effect in 2007. Under this Pediatric Regulation, pharmaceutical companies are now required to perform studies in pediatric patients, and include the data in the submission file, when applying for a new marketing authorization or when applying for the addition of a new indication in the label for an already approved compound. The planned work in pediatric patients needs prior approval from the Pediatric Committee (PDCO) of the European Medicines Agency (EMA). This enables the PDCO to review the pediatric investigation plan (PIP), and where applicable request amendments to be made. In case the drug is irrelevant to the pediatric population, the PDCO may issue a waiver

at the request of the applicant. Also the pediatric studies may be postponed by issuing a deferral, for example if safety of a compound has been insufficiently established in adults and administration to children can thus not be justified. Any application for a marketing authorization will not be accepted by the EMA if it is not accompanied by results from studies approved in a PIP (unless a deferral or waiver was issued).

The above measure affects only newly marketed compounds. If pharmaceutical companies submit data complying with an approved PIP, they will receive a 6 month extension to the Supplementary Protection Certificate (SPC). This SPC extension effectively is an extension to the product patent, banning generic companies from entering the market for an additional 6 months and thereby creating substantial extra revenue to the applicant.

The results of this legislation are quite modest, but have led to a small increase in the proportion of clinical trials performed in the pediatric population (6). As the authorities can only offer powerful financial incentives to industry for new drugs covered by a patent, these trials are mainly performed with relatively new compounds. We have previously shown that research is primarily driven by drugs generating large revenue, rather than by drugs actually used by children or important for children in general (7). The additional revenue for pharmaceutical companies is quite substantial: the economic impact of patent extension of statins, ACE inhibitors and SSRI's under similar legislation in the United States was estimated at 430 million US dollars (8). This figure is not available for Europe, but it is likely to be of a similar magnitude.

The compounds used most often by children, for example anti-microbial drugs, topical steroids and bronchodilators (9), are mostly off-patent and therefore lack an adequate financial incentive for performing research and submitting the acquired data to the competent authorities. This fact has been recognized both in the U.S. and in Europe, with respectively the FDA and the EMA drawing up priority lists for medicines requiring research. In an attempt to provide financial incentive for companies submitting data, applicants can earn 10 years of market exclusivity for formulations intended for children under the so-called Pediatric Use Marketing Authorization (PUMA). In addition, the authorities will provide financial support for research on compounds on the priority list, with funds being channeled through the National Institute of Health (NIH) in the United States, and through the 7th Framework Program of the European Commission in Europe.

To accommodate the increasing amount of research being performed with children, a myriad of networks has been erected concentrating knowledge and expertise on pharmacological research involving children. Examples include the NIH funded Pediatric Pharmacology Research Units in the United States, the Medicines for Children Research Network (MCRN) in the United Kingdom, and disease specific networks such as the Children's Oncology Group in the U.S., the Innovative Therapies for Children with Cancer (ITCC) consortium in Europe, and the Pediatric Rheumatology International Trials Organisation (Printo) in Europe. In Europe, a 'network of networks' was established in accordance with the 2006 Pediatric Regulation. This European Network of Paediatric Research at the European Medicines Agency (Enpr-EMA) aims to enhance collaboration between the existing European networks.

In an attempt to reduce the risks associated with off-label prescribing, national pediatric formularies have been set up in many countries, offering consensus- and if possible evidence-based guidance in drug prescription for children. In the Netherlands this national formulary was set up as an initiative of its national medicines for children research network. It is currently directly government-funded and has been adopted by both the Paediatric Association of the Netherlands and the pharmacists' associations as a formal guideline (10). It is however still important to address the government in such a way that she will continue the financial support for this unique project in the forthcoming years.

Ethics and regulatory approval

Another hurdle in performing research with children is formed by the fact that non-therapeutic research in minors may not include more than minimal risk and burden for participants in this research. Where it is acceptable to perform dose-finding and toxicity (Phase 1) research in adults, and subjecting adults to invasive procedures in evaluating drug effects, this is not acceptable in children as they, being a minor, are viewed as being incapable to provide informed consent.

In the Netherlands, several stakeholders have argued that these restrictions are too stringent, and that exceptions to the rule should be made possible. For example, pediatric oncologists have stated that these limitations restrict access to new and adequately researched compounds (11). The Dutch Central Committee on Research Involving Human Subjects, in its annual report 2006, has also called for reconsideration of the current legislation, as the committee felt that it is currently forced to reject potentially important studies. The interpretation of minimal risk and burden, as well as the question in what situations this threshold of risk and burden may be surpassed, are a subject of large debate on a national level. Anna Westra has proposed a few options in her recent thesis (2011) on the interpretation of minimal risk and burden, which can lead to a practical solution in this field. The Netherlands are currently in the process of revising applicable legislation (12).

Outline of this thesis

It is a large challenge and sometimes requires a great deal of creativity to design adequate trials involving children. Apart from being scientifically sound, the trial design needs to consider the special position of children by minimizing risks and burden to the participants. In order to reach this goal, investigators should (1) search for situations where data can be gathered without creating burden (for example: taking blood samples when an IV cannula is already in place for therapeutic reasons) and (2) develop minimally invasive methodology that can be used in the evaluation of the pharmacokinetics and pharmacodynamics of the researched compound.

This thesis covers some examples of how effective pharmacological research can be performed, without creating more than minimal risk or burden to the participating children. In Chapter 2 we have assessed and commented upon the effects of several years of the Pediatric Rule in the United States. In Chapter 3, we have investigated whether crushing and dissolving levothyroxine tablets before administration using a nasogastric feeding tube may lead to dosing errors. Chapter 4 covers the clonidine test (a diagnostic procedure for assessing the integrity of the growth-hormone axis). This procedure is associated with moderately severe side-effects, however the pharmacokinetic profile of clonidine during the test procedure is not known. Chapters 5 and 6 cover work with a neurocognitive test battery which has been extensively validated in adults. In Chapter 5, we investigated whether healthy children would be able to complete the tasks and how they tolerated performing these tasks. In Chapter 6, we investigated whether the results of these tasks could be used as biomarker for drug effects in children with ADHD. In addition, saliva sampling was performed for pharmacokinetic analysis of methylphenidate levels. Finally, in Chapter 7 a bedside device for determination of the activated partial thromboplastin time (APTT) is evaluated. If effective, this device could lead to a reduction in burden of a diagnostic procedure by reducing the amount of blood required for determination of the APTT from a tube to a drop.

1 Choonara I, Conroy S. Unlicensed and off-label drug use in children: implications for safety. Drug Saf. 2002;25(1):1-5.

2 Lindell-Osuagwu L, Korhonen MJ, Saano S, Helin-Tanninen M, Naaranlahti T, Kokki H. Off-label and unlicensed drug prescribing in three paediatric wards in Finland and review of the international literature. J Clin Pharm Ther. 2009;34(3):277-87.

3 Sutherland JM. Fatal cardiovascular collapse of infants receiving large amounts of chloramphenicol. AMA J Dis Child. 1959;97(6):761-7.

4 Lenz W. Epidemiology of Congenital Malformations. Ann N Y Acad Sci. 1965;123: 228-36.

5 Steinbrook R. Testing medications in children. N Engl J Med. 2002;347(18):1462-70. DOI 10.1056/NEJMhpr021646

6 Olski TM, Lampus SF, Gherarducci G, Saint Raymond A. Three years of paediatric regulation in the European Union. Eur J Clin Pharmacol. 2011;67(3):245-52. DOI 10.1007/s00228-011-0997-4

7 Boots I, Sukhai RN, Klein RH, Holl RA, Wit JM, Cohen AF, et al. Stimulation programs for pediatric drug research--do children really benefit? Eur J Pediatr. 2007;166(8):849-55. DOI 10.1007/s00431-006-0381-z

8 Nelson RE, McAdam-Marx C, Evans ML, Ward R, Campbell B, Brixner D, et al. Patent extension policy for paediatric indications: an evaluation of the impact within three drug classes in a state Medicaid programme. Appl Health Econ Health Policy. 2011;9(3):171-81. DOI 10.2165/11539060-000000000-00000

9 Chai G, Governale L, McMahon AW, Trinidad JP, Staffa J, Murphy D. Trends of Outpatient Prescription Drug Utilization in US Children, 2002-2010. Pediatrics. 2012. DOI 10.1542/peds.2011-2879

10 Off-label prescription of medication by the paediatrician (Dutch), Paediatric Association of the Netherlands. 2009.

11 Verschuur AC, Zwaan CM. Nederland kan niet achterblijven. Fase-1 onderzoek noodzaak voor kinderen met kanker. Medisch Contact. 2007;62(21):909-12.

12 Westra AE. Wetenschappelijk onderzoek met kinderen: maak de regels niet te ruim. [Medical research in children: should the rules be eased?]. Ned Tijdschr Geneeskd. 2010;154:A2275.

CHAPTER 2

Stimulation programs for pediatric drug research – do children really benefit?

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Abstract

BACKGROUND Most drugs that are currently prescribed in pediatrics have not been tested in children. Pediatric drug studies are stimulated in the USA by the pediatric exclusivity provision under the Food and Drug Administration Modernization Act (FDAMA) that grants patent extensions when pediatric labeling is provided. We investigated the effectiveness of these programs in stimulating drug research in children, thereby increasing the evidence for safe and effective drug use in the pediatric population.

METHODS All drugs granted pediatric exclusivity under the FDAMA were analyzed by studying the relevant summaries of medical and clinical pharmacology reviews of the pediatric studies, or if these were unavailable the labeling information as provided by the manufacturer. A systematic search of the literature was performed to identify drug utilization patterns in children.

RESULTS From July 1998 to August 2006, 135 drug entities were granted pediatric exclusivity. Most frequent drug groups were anti-depressants and mood stabilizers, ACE inhibitors, lipid-lowering preparations, HIV antivirals and non-steroidal anti-inflammatory and anti-rheumatic drugs. The distribution of the different drugs closely matched the distribution of these drugs over the adult market, and not the drug utilization by children.

CONCLUSIONS Many drug studies in children have been performed since the introduction of the FDAMA; however, children infrequently use the drugs granted pediatric exclusivity. The priorities for pediatric drug research should be set by the need of the patients, not by market considerations.

Introduction

Most drugs prescribed in pediatrics have not been tested in children. A recent review demonstrates that up to 80% of prescriptions for children in hospital and in the general practice are either unlicensed (without a license for children) or used off-label (outside the product license) (1). Of commercially available drugs in Europe, only 35% are authorized for use in children (2). Although there are reasons why children do not often participate in clinical trials, including ethical, scientific and commercial considerations (3, 4), it is considered unacceptable to treat children with drugs that have not been studied properly.

In 1997 the Food and Drug Administration (FDA) and the Congress introduced the Food and Drug Administration Modernization Act (FDAMA) and this was followed by the Best Pharmaceuticals for Children Act. Closely linked to this legislation is the Pediatric Rule (1998), which requires the industry to perform research in the pediatric population. In the European Union (EU) final legislation on this topic has been approved, and will come into effect in the beginning of 2007.

In both continents the measures taken to address the problems broadly follow the same pattern. The first incentive is aimed at new medicines and intended for products covered by a patent or a supplementary protection certificate (SPC). For these drugs a six-month extension of market exclusivity is granted if a pediatric study is performed. The second incentive has the objective to increase the knowledge on drugs that are not longer patent protected. In order to obtain the data necessary to establish safety, quality and efficacy specifically in children, either funding for the studies (USA) or market exclusivity (a so-called Pediatric Use Marketing Authorization-PUMA) can be given. Central in the second incentive is that experts are involved to determine for which drugs the greatest medical need exist and that these drugs will be given priority. In the USA the FDA plays this central role and in the EU a Pediatric Committee will be established within the European Medicines Agency (EMEA) which will be given a similar role.

The EU Commission apparently follows the approach in the USA with regard to patent-protected drugs because according to the EU Commission 'the pediatric exclusivity provision has been extremely successful in the USA in stimulating the development of medicinal products for pediatric use'(5).

At the brink of implementing new programs (EU) or decisions on continuation of existing programs (USA), we questioned what the influence of the pediatric exclusivity regulation has been on pediatric drug development. We evaluated the drugs that are granted pediatric exclusivity in the USA, by studying research that has been performed as a consequence of the exclusivity provision, and by comparing the drugs granted pediatric exclusivity with medicines actually used by children.

Methods

The drugs granted pediatric exclusivity since the introduction of the Food and Drug Administration Modernization Act in July 1998 until August 2006, were retrieved from the FDA-website (6). All drugs were classified into subgroups according to the Anatomical Therapeutical Classification (ATC) system of the European Pharmaceutical Marketing Research Association. The data submitted to the FDA that resulted in granting the exclusivity, were examined. For each drug, the publicly available summaries of medical and clinical pharmacology reviews of the pediatric studies were scrutinized. These summaries contain information on the pediatric research performed to obtain pediatric exclusivity. If these summaries were unavailable, the labeling information of the drug with pediatric exclusivity was studied to retrieve information about the pediatric studies performed. Each drug label contains a pediatrics section stating whether the drug is tested in children and if so, information on the performed studies is given (6-8). Data were extracted about the type of study, number and age of participants, whether long-term follow-up (defined as >1 year with specific attention for long term effects on growth and development) was undertaken and whether the study led to a pediatric indication being included in the label. Participants were divided into pediatric age categories according to the International Conference on Harmonization (ICH) guidelines: neonates (birth–27 days), infants (28 days-23 months), children (2–11 years) and adolescents (12–18 years).

To obtain data about drug use in children, recently published surveys of drug prescribing in hospitals and general practice were reviewed by a systematic search of the literature on drugs used by children. Details on the literature search and selection criteria are provided in box 1. Drug use in adults was assessed by using the sales figures from public databases and publications thereof (9, 10). The data were categorized according to the ATC system as mentioned above.

Results

PEDIATRIC EXCLUSIVITY PROVISION

According to the FDA 135 drugs (130 active moieties) were granted pediatric exclusivity from July 1998 to August 2006. Most frequent drug groups were central nervous system drugs (19%), such as anti-depressants and psycholeptics, cardiovascular drugs (16%), mainly ACE inhibitors and lipid-lowering preparations, systemic anti-infectives (12%), among which largely HIV antivirals, cytostatics (11%), and alimentary tract medication (12%), among which proton pump inhibitors and oral antihyperglycemic medication (table 1).

From 118 drugs (91%) information about studies performed in children to obtain pediatric exclusivity could be retrieved from the FDA summaries of medical and clinical pharmacology reviews (n=61) of the pediatric studies or the prescribing information as provided by the manufacturer (n=57). No information could be found on 12 of the drug entities, mainly including over the counter drugs. The product label of 13 drugs merely

stated that safety and/or efficacy in the pediatric population had not been established. For the remaining 105 drugs, in total, 326 studies were performed for the approval of pediatric exclusivity. At least 40,075 pediatric patients participated in these 326 studies (The number of participants was not noted for 27 of these studies). In the majority of the applications children over a wide age range were included. Information on the age of participants was not provided for 54 of the 326 studies. Children participated in 224 of the remaining 272 studies (82%), adolescents in 177 (65%), infants were included in 105 (39%) of the trials and neonates in 38 trials (14%). Only 1 (0,4%) study included preterm infants. The objective of most (62%) of the 326 studies was to determine the safety and efficacy of a drug. Pharmacokinetics and/or pharmacodynamics were studied in 147 (45%) trials, efficacy-only in 13 (4%) and safety-only in 38 (12%). From the 299 studies in which information about the number of participants was given, on average 134 children participated per study. This varied from 8 children in a safety and efficacy study of a HIV antiviral in neonates, to 994 children participating in a safety database of an antibiotic. Long-term (>1 year) follow-up was either done or planned for 25 (21%) of the 118 drugs.

For 42% of the drugs granted pediatric exclusivity and for which information was available (50 out of 118), the information obtained from the pediatric studies led to an approved pediatric indication.

The 130 active moieties granted pediatric exclusivity were categorized according to the ATC system. The results are shown in table 1.

DRUG UTILIZATION BY CHILDREN AND ADULTS

The search strategy retrieved 9 papers addressing drug utilization in children (table 2). The data were summarized by calculating a weighted average for each drug category with weights proportional to the number of prescriptions (table 3). Drugs most often used by children are respiratory drugs, anti-infectives for systemic use and dermatologicals.

The pattern of drug use in adults based on sales over the last 12 months to May 2005 (table 4) shows that drugs used for central nervous system, cardiovascular, alimentary tract and metabolism disorders make up over 50% of the market.

Discussion

The introduction of the pediatric exclusivity laws in the USA has led to pediatric drug trials for 135 drugs that were tested in the past 8 years. Over 300 studies were performed with over 40,000 pediatric patients participating. Based on these data the FDA claims 'the pediatric exclusivity provision has done more to generate clinical studies and useful prescribing information for the pediatric population than any other regulatory or legislative process to date' (11). The EU Commission and other policy makers see a rapid adaptation of similar legislation in Europe essential to boost pediatric drug research in the EU as soon as possible (5, 12, 13). Although it is true that more pediatric studies were done, we question the content of the research.

A major discrepancy is apparent between the drug prescription pattern in children and the drugs granted pediatric exclusivity. The majority of drugs granted pediatric exclusivity is rarely used by children and drugs that are frequently used by children are underrepresented in the pediatric studies to obtain exclusivity. This difference is not accounted for by the licensing status of the used drugs. For instance, in general practice 37% of the prescriptions of respiratory drugs are still used in an unlicensed or off-label manner (14). This suggests that the pediatric studies did not address the real needs in pediatric drug development. Whilst the pediatric exclusivity scheme has stimulated pediatric research quantitatively, the nature of the scheme has led to priorities for the type of research that are largely driven by the adult market for medicines rather than by the needs of the pediatric population. An important built-in motive in the stimulation

program for performing pediatric studies is the financial benefit gained from the exclusivity provision. The drugs granted pediatric exclusivity include 5 out of the 'Top 10' prescription drugs with the highest sales figures in North-America in 2005: atorvastatin (Lipitor®), simvastatin (Zocor®), omeprazole (Nexium®), Lansoprazol (Prevacid®) and sertraline (Zoloft®). Sales of these 5 drugs added to 24.1 billion US dollar in 2005 (15). Extension of the SPC on the basis of pediatric exclusivity has also been granted to other adult best-seller drugs, such as pravastatine, enalapril, metformin, amlodipine, paroxetine, fluoxetine and rofecoxib (currently withdrawn). It is thus clear that the patent extension has an enormous financial benefit for the pharmaceutical companies, which easily outweighs the cost of the average pediatric trial (5). This is not only the case for bestseller drugs but also for drugs with a smaller market share. A Tufts University report indicates that an estimated 35 million US dollar in undiscounted profits per drug can be earned by obtaining pediatric exclusivity based on median 2004 sales for all pediatric exclusivity drugs after accounting for costs and market protection extensions (16). Although the increased profit was intended by the legislator we doubt if the other intention, which aimed at providing data to reduce the volume of drugs taken by children that are off-label or unlicensed, has also been met.

Our approach to define essential drugs for children by volume and by number of children that need a certain medicine is incomplete as also indications and severity of the condition for which the drug is prescribed play an important role (17). Secondly, the method employed in our literature search may have introduced some bias toward outpatient drug consumption, thus underestimating the use of for example anesthetic or cardiovascular drugs. Clearly, hypertension or lipid disorders occur in children and knowledge about these drugs in this population is important. However, these conditions are certainly not major causes of morbidity and subsequent drug use in children and the research priorities lie elsewhere.

Furthermore there are specific issues in pediatric clinical pharmacology that have remained unaddressed by the current product-related approach. First, the age distribution of the children participating in the trials does not reflect the actual medical needs. Drug use in children follows a pattern with a relatively high percentage of children using a prescription drug in the first year of life (18) and the proportion of off-label and unlicensed drug use is highest (~90%) in the vulnerable group of neonatal ICU patients (19). These data are not reflected in the participation in the trials to obtain pediatric exclusivity, with neonates and infants being included in respectively 14 and 39% of the study groups. In fact it is surprising to see that the majority of applications included children of a wide age range, thereby ignoring the delineation of age groups as defined in the ICH-guidelines. This is problematic, as in the pediatric population significant age-related differences may exist in the pharmacokinetics and the effects of drugs (20). Secondly, the effects of drugs (that are intended for chronic use) on development and maturation of children are largely unknown, but this issue has hardly been addressed. Third, off-label drugs are hardly or not studied. Only 10 drugs in this category are under consideration for study by pharmaceutical companies (FDA website). From these 10 drugs, 6 drugs already have been refused by industry for studying, and have been referred to the National Institutes of Health to obtain the necessary information for rational use in pediatric populations. These clinical trials are at the moment awaiting funding (21, 22). Surprisingly, in the same Tufts study that reports on a profit of 35 million US dollars per drug granted exclusivity, it is also suggested that the current incentives for studying non-patent protected drugs are too low (16).

Finally, methodological research has remained underfunded by this approach. Any expansion of research will require specialized techniques that allow samples and data to be obtained in children with minimal discomfort and risk. For instance, most pharmacokinetic assays require an amount of blood that is too large for the average neonate, so highly sensitive assays need to be developed. Accurate assessment of drug effects on neuro-development and behavior also require further development, independently of studies with particular compounds.

The recently approved EU guidelines contain differences from the USA that may remedy some of the deficiencies indicated. It contains a section for the stimulation of off-patent drugs that can be granted a special label (PUMA) and data protection if data necessary to establish safety, quality and efficacy in children are submitted. The 'Medicines Investigation for the Children of Europe'-program, funded by the EU, will be created to stimulate research of off-patent drugs. The proposed establishment of a Pediatric Committee, operating within the European Medicines Agency, will guard study of significant drugs for children and avoid unnecessary studies, provide free advice to industry and stimulate long-term pharmacovigilance. It will also play a role in the implementation of the requirement for industry to submit data they already hold on use of their medicines in children. New drugs will not be granted Marketing Authorisation unless the need for pediatric research has been waived by the Pediatric Committee, or deferral of initiation or completion of an already approved Pediatric Investigation Plan has been agreed to by this committee. In addition, an EU network of investigators and trial centers will be formed (5). Although elements of this network are now beginning to form, their funding is unclear and certainly not at the level provided to the patent holders by a patent extension. Also, it is not immediately clear why the EU measures will deliver, while this was not achieved with the formation of Pediatric Pharmacology Research Units and the oversight of research priorities by the FDA in the USA.

In conclusion, the schemes implemented in the US generated new knowledge and led to the rapid development of an infrastructure to carry out pediatric drug trials. Although these are certainly positive developments, we do believe that the findings of this survey warrant additional efforts to stimulate research on drugs used more frequently by children and generally applicable methodological research, as at least the short-term effect of these initiatives seems to have drawn the focus of industry-sponsored research to the most profitable part of the market. The funding of the research we propose should not be problematic.

The pediatric exclusivity schemes generate a flow of public money to the sponsoring drug companies because generic replacement and price reductions are postponed. There is no particular reason why these public funds could not be at least partly applied in an alternative manner.

BOX 1 Literature search and selection strategy

STEP 1	Literature searches of the PubMed and Embase bibliographic databases for papers published in English between 1990 and July 2005 combining the following search terms (keywords and appropriate medical subject headings): *child, preschool (or) child (or) infant (or) infant, newborn (or) adolescent (or) pediatric (or) paediatric (or) paediatrics; pharmaceutical preparations (or) drugs, non-prescription (or) drugs, generic (or) drug, therapy (or) prescriptions, drug (or) medicine (or) medication; drug utilization (or) pharmacoepidemiology (or) drug utilization review*
STEP 2	The references contained in articles identified in step 1 were examined to identify further relevant studies
STEP 3	Based on the titles and abstracts of the papers, we next identified and then located full-text copies of 35 potentially relevant studies for closer examination
STEP 4	Selection of articles by two independent reviewers (IB, RS), using the following selection criteria: *Study performed in the industrialized world, defined as in Europe, North America, Australia and New Zealand; Study population of children from 0 to at least 14 years, to include all age groups (neonates, infants, children and adolescents); Sufficient information in the paper about drug utilization to classify 90% of the drugs in an atc drug groups; Avoidance of selection bias by exclusion of voluntary surveys*
STEP 5	Inclusion of 9 papers meeting all selection criteria

TABLE 1 Active moieties granted pediatric exclusivity according to ATC group

Drug Category	Number of drugs (% of total)	Most frequent drug classes	Number of drugs
Central nervous system	24 (19%)	Anti-depressants	8
		Psychostimulants, agents used for ADHD and nootropics	4
		Anti-epileptics	3
		General Anaesthetics	3
		Anti-migraine preparations	2
Cardiovascular system	21 (16%)	ACE inhibitors, plain	6
		HMG-CoA reductase inhibitors	5
		Beta-blocking agents	4
Alimentary tract and metabolism	16 (12%)	Drugs for peptic ulcer and gastro-oesophageal reflux disease	5
		Oral blood glucose lowering drugs	4
Anti-infectives for systemic use	15 (12%)	Direct acting antivirals	10
		Antibacterials for systemic use	4
Antineoplastic and immuno-modulating agents	14 (11%)	Other antineoplastic agents	5
		Antimetabolites	3
		Alkylating agents	2
Musculo-skeletal system	7 (5%)	Anti-inflammatory and anti-rheumatic products, non-steroids	6
		Drugs affecting bone structure and mineralization	1
Respiratory system	10 (8%)	Antihistamines for systemic use	4
		Drugs for obstructive airway disease	3
		Decongestants and other nasal preparations for topical use	2
Sensory organs	10 (8%)	Antiglaucoma preparations and miotics	4
		Decongestants and anti-allergics	3
Dermatologicals	6 (5%)	Corticosteroids, potent (group 3)	3
Genito-urinary system and sex-hormones	3 (3%)	Other urologicals, including antispasmodics	2
Blood and blood forming organs	2 (2%)	Anti-thrombotic agents	1
Systemic hormonal preparations	1 (1%)	Hypothalamic hormones	1
Parasitology	1 (1%)	Anti-malarials	1
Total	130 (100%)		

TABLE 2 Characteristics of included studies on pediatric drug utilization

Author (year)	Setting	Population size	Age range (yrs)	Number of prescriptions
Niclasen (23) (1995)	Pharmacy dispensing records in – and outpatients	1704	0-14	5876
Rokstad (24) (1997)	Prescriptions of general practitioners	†	0-19	8215
Thrane (25) (1999)	Pharmacoepidemiological prescription database outpatients	48091	0-15	154189
Schirm* (18) (2000)	Pharmacy dispensing records outpatients	15001	0-16	373925
Lewis (26) (2001)	Prescriptions pediatric outpatients	12628	0-16	33140
Pandolfini (27) (2002)	Prescriptions general pediatric hospitals	1325	0-14	4265
Schirm (28) (2003)	Pharmacy dispensing records outpatients	18943	0-16	66222
Ufer (29) (2003)	Pharmacy dispensing records outpatients	357784	0-16	644817
Sanz (30) (2004)	Prescriptions outpatients by GPs and pediatricians	12264	0-14	27486

*not included in weighted average due to presentation of data in paper (percentage of children using a drug category);
† not stated

TABLE 3 Drug utilization pattern in children

Drug Category	% of prescriptions	Frequently used drug classes
Respiratory system	30 %	Drugs for obstructive airway disease
		Nasal preparations
		Cough and cold preparations
		Antihistamines for systemic use
General anti-infectives, systemic	28%	Antibacterials for systemic use
Dermatologicals	12%	Antifungals for dermatological use
		Emollients and protectives
		Corticosteroids, dermatological preparations
Sensory organs	7%	Ophtalmologicals
		Otologicals
Central nervous system	4%	Analgesics/ antipyretics
		Psychostimulants, agents used for ADHD and nootropics
		Anti-epileptics
Hormones	3%	Corticosteroids, for systemic use
Blood and blood forming organs	3%	Vitamin K
Alimentary tract and metabolism	2%	Drugs for peptic ulcer and gastro-oesophageal reflux disease
		Insulins and analogues
Musculo-skeletal system	2%	Anti-inflammatory and anti-rheumatic products, non-steroids
Genito-urinary system and sex-hormones	2%	Hormonal contraceptives for systemic use
Cardiovascular system	1%	Diuretics
Other	<1%	
Total	105% (exceeds 100% due to rounding)	

TABLE 4 Drug utilization patterns in adults, according to the drug sales in North America over the period May 2004-May 2005

Drug Category	Adult prescriptions
Central nervous system	23%
Cardiovascular system	19%
Alimentary tract and metabolism	14%
Respiratory system	9%
General anti-infectives, systemic	8%
Musculo-skeletal system	6%
Genito-urinary system and sex-hormones	6%
Other	2%
Antineoplastic and immunomodulating agents	4%
Dermatologicals	3%
Blood and blood forming organs	3%
Sensory organs	2%
Total	100%

1 Pandolfini C, Bonati M. A literature review on off-label drug use in children. Eur J Pediatr. 2005;164(9):552-8. DOI 10.1007/s00431-005-1698-8

2 Ceci A, Felisi M, Catapano M, Baiardi P, Cipollina L, Ravera S, et al. Medicines for children licensed by the European Agency for the Evaluation of Medicinal Products. Eur J Clin Pharmacol. 2002;58(8):495-500. DOI 10.1007/s00228-002-0511-0

3 Caldwell PH, Murphy SB, Butow PN, Craig JC. Clinical trials in children. Lancet. 2004;364(9436):803-11. DOI 10.1016/S0140-6736(04)16942-0

4 Smyth RL, Weindling AM. Research in children: ethical and scientific aspects. Lancet. 1999;354 Suppl 2:SII21-4.

5 Arlett P. Proposed regulation on medicinal products for paediatric use. Overview and explanation of the proposal. http://pharmacos.eudra.org/F2/Paediatrics/docs/overview and explanation of the proposal 1.pdf. 2004 [accessed July 27, 2005].

6 Pediatric Exclusivity Labeling Changes. http://www.fda.gov/cder/pediatric/labelchange.htm. 2006 [accessed August 23, 2006].

7 Summaries of Medical and Clinical Pharmacology Reviews of Pediatric Studies. http://www.fda.gov/cder/pediatric/Summaryreview.htm. 2005 [accessed August 23, 2006].

8 Drugs@FDA. http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm. 2006 [accessed August 23, 2006].

9 IMS World Review 2004. IMS, Fairfield, Connecticut, USA, 2004.

10 IMS Health Retail Drug Monitor. IMS, Fairfield, Connecticut, USA, 2005.

11 Roberts R, Rodriguez W, Murphy D, Crescenzi T. Pediatric drug labeling: improving the safety and efficacy of pediatric therapies. JAMA. 2003;290(7):905-11. DOI 10.1001/jama.290.7.905

12 Proposal for a regulation of the European parliament and of the council on medicinal products for paediatric use and amending Regulation (EEC) No 1768/92, Directive 2001/83/EC and Regulation (EC) No 726/2004, Commission of the European communities, 2004.

13 Ramet J. What the paediatricians need--the launch of paediatric research in Europe. Eur J Pediatr. 2005;164(5):263-5. DOI 10.1007/s00431-005-1633-z

14 t Jong GW, Eland IA, Sturkenboom MC, van den Anker JN, Strickerf BH. Unlicensed and off-label prescription of respiratory drugs to children. Eur Respir J. 2004;23(2):310-3.

15 IMS National Sales Perspectives. IMS Health, Fairfax, Connecticut, USA, 2006.

16 Tufts Center for the Study of Drug Development. U.S. pediatric studies incentive led to new labeling for nearly 100 drugs – Impact Report 2005;7(4).

17 Kauffman RE. Essential drugs for infants and children: North American perspective. Pediatrics. 1999;104(3 Pt 2):603-5.

18 Schirm E, van den Berg P, Gebben H, Sauer P, De Jong-van den Berg L. Drug use of children in the community assessed through pharmacy dispensing data. Br J Clin Pharmacol. 2000;50(5):473-8.

19 Conroy S, McIntyre J, Choonara I. Unlicensed and off label drug use in neonates. Arch Dis Child Fetal Neonatal Ed. 1999;80(2):F142-4; discussion F4-5.

20 Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental pharmacology--drug disposition, action, and therapy in infants and children. N Engl J Med. 2003;349(12):1157-67. DOI 10.1056/NEJMra035092

21 U.S. department of health and human services, National Institutes of Health (NIH), National Institute of Child Health and Human Development (NICHD). Progress in Implementing the Best Pharmaceuticals for Children Act (BPCA). http://www.nichd.nih.gov/bpca/documents/progress_implementing_BPCA.pdf. 2005 [accessed August 20, 2005].

22 Murphy D. The role of the FDA in pediatric research [presentation], presented at the Glaser Pediatric Research Network Symposium. Boston, USA: 2004.

23 Niclasen BV, Moller SM, Christensen RB. Drug prescription to children living in the Arctic. An investigation from Nuuk, Greenland. Arctic Med Res. 1995;54 Suppl 1:95-100.

24 Rokstad K, Straand J, Fugelli P. General practitioners' drug prescribing practice and diagnoses for prescribing: the More & Romsdal Prescription Study. J Clin Epidemiol. 1997;50(4):485-94.

25 Thrane N, Sorensen HT. A one-year population-based study of drug prescriptions for Danish children. Acta Paediatr. 1999;88(10):1131-6.

26 Lewis MA, Kuhl-Habich D, von Rosen J. Drug use and adverse event monitoring in German children. Int J Clin Pharmacol Ther. 2001;39(11):507-12.

27 Pandolfini C, Impicciatore P, Provasi D, Rocchi F, Campi R, Bonati M. Off-label use of drugs in Italy: a prospective, observational and multi-centre study. Acta Paediatr. 2002;91(3):339-47.

28 Schirm E, Tobi H, de Jong-van den Berg LT. Risk factors for unlicensed and off-label drug use in children outside the hospital. Pediatrics. 2003;111(2):291-5.

29 Ufer M, Rane A, Karlsson A, Kimland E, Bergman U. Widespread off-label prescribing of topical but not systemic drugs for 350,000 paediatric outpatients in Stockholm. Eur J Clin Pharmacol. 2003;58(11):779-83. DOI 10.1007/s00228-003-0560-z

30 Sanz E, Hernandez MA, Ratchina S, Stratchounsky L, Peire MA, Lapeyre-Mestre M, et al. Drug utilisation in outpatient children. A comparison among Tenerife, Valencia, and Barcelona (Spain), Toulouse (France), Sofia (Bulgaria), Bratislava (Slovakia) and Smolensk (Russia). Eur J Clin Pharmacol. 2004;60(2):127-34. DOI 10.1007/s00228-004-0739-y

CHAPTER 3

Levothyroxine for neonates and children with a nasogastric feeding tube: solution for drops superior to tablets

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Abstract

Treatment of hypothyroidism in newborns, infants, and children fed through nasogastric feeding tubes may lead to practical difficulties because levothyroxin is only marketed in tablet formulations in The Netherlands. Crushing these tablets and mixing the obtained powder with water results in a suspension with poorly dissolved levothyroxin. After administering this suspension, a residue will remain in the nasogastric feeding tube or spoon used. This may lead to decreased availability and practical difficulties when administering the drug. In surrounding EU countries, a liquid formulation of levothyroxin is commercially available. Therefore, we recommend the use of levothyroxin drops for newborns, infants or patients with a nasogastric feeding tube.

Introduction

Hypothyroidism is a medical condition that is relatively common, with an estimated prevalence of 0.1-2% of children in western society (1). This includes the acquired forms such as Hashimoto's disease and the congenital forms of hypothyroidism. Treatment of this condition consists of administration of levothyroxine. Although levothyroxine is available as a solution in several European countries, e.g. L-Thyroxine SERB in France, Evotrox in Great Britain and L-thyroxine Henning in Germany, only tablets are marketed in the Netherlands (2). In the pediatric age groups, this frequently leads to practical problems, as infants are not able to ingest these tablets. Common practice is to circumvent this by administering crushed tablet with water on a spoon, in accordance with Dutch practice guidelines (1). The first edition of this guideline recommended using a metal spoon, later editions explicitly stated that plastic spoons were also suitable. The latest (4th) edition states that plastic syringes should not be used. Although any interaction between levothyroxin and plastics has not been reported in the medical literature, the explicit mentioning of materials to be used did raise some practical questions in our clinic. Exposure of levothyroxine to plastic surfaces is frequently inevitable in the clinical setting, for example when it is administered through a nasogastric feeding tube (NFT). Adsorption of strongly lipophilic compounds such as ciclosporin or diazepam to PVC is a known issue, but to our knowledge this phenomenon has not been associated with levothyroxine. The chemical properties of levothyroxine do not suggest he possibility of an interaction with plastics (3).

In additional issue in current practice is that the preparation of a liquid from tablets needs to be performed in clinical departments or even in the home setting. This is done by crushing the tablets and mixing it with water. A standardized procedure is not available, and intended concentrations of the prepared liquid are often above the possible solubility of levothyroxine. Preparation of a true solution is therefore not possible.

The use of a suspension is discouraged in the current practice guideline, as it may lead to preparation and dosing errors (1).

To come to an evidence based advice on how to administer levothyroxine in an adequate manner, we performed a study in the pharmacy laboratory. In this study we studied 1) the solubility of levothyroxine in water and 2) the exposure of levothyroxine containing liquids tot plastic surfaces, in this case NFT's.

Methods

In the pharmacy laboratory, several levothyroxine solutions were prepared. Using an ultrasonic bath, 25 mcg tablets were dissolved in water and in 4.2% sodium bicarbonate (pH ~ 8). With European standard reference L-thyroxine (Ph Eur) a reference solution was prepared (pH~10). In all cases, the intended concentration reached would be 15 mcg/ml if the tablets would fully dissolve.

The obtained drug solutions were instilled in a polyurethane NFT (Nutrisafe 2, Ch. 8, 130 cm. Vygon, Valkenswaard, NL) for a period of one hour. After an hour the NFT was emptied by flushing it with 50 cc's of air.

Both before and after instillation in the NFT, the obtained solutions were centrifuged and subsequently analyzed using reverse phase-high pressure liquid chromatography (RP-HPLC, method validation on file). On a later moment, the experiment was repeated with the solution commercially available in Germany* (L-thyroxin Henning Tropfen 100 mcg/mL, Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany).

All experiments were performed in pairs, the average measurement result was used in the results section.

Results

The measured concentrations of levothyroxine of the 4 solutions that we used in our study are presented in Table 1. The loss of drug after instillation in a NFT is shown in percentage of the initial concentration.

** The German product was used as it was available through the international pharmacy in Venlo. We have no reason to believe that the English product Evotrox® or the French product L-Thyroxine SERB are not suitable.*

A remarkable finding is that the intended concentration of 15 mcg/ml in the prepared solution was not reached due to unexpectedly poor solubility of levothyroxine at pH levels <10. The commercial solution had a concentration of 100 mcg/ml.

Discussion

We have evaluated the solubility of levothyroxine tablets in several solutes (water and sodium bicarbonate 4.2%). In addition, the degree to which levothyroxine from these different solutions remains in a polyurethane NFT after a 1 hour dwell time was evaluated. The latter experiment was repeated with a preparation available from a commercial party.

A surprising finding was the fact that levothyroxine (-sodium) has a very poor solubility at normal pH values, lower than described in manuals such as the Merck index. Also at a pH of 8 levothyroxine was found not to become dissolved. This means that these preparations should be described as suspensions rather than solutions. After a 1-hour dwell time in a polyurethane NFT, considerable amounts of levothyroxine were found to remain in the NFT (22 and 23%, respectively). Comparison with a reference solution and a commercially available product from Germany showed only minimal (2%) loss after instillation in the NFT. The latter figure rules out adsorption of levothyroxine to the polyurethane surface, and rather suggests that the loss of levothyroxine should be explained by non-dissolved tablet particles staying behind in the NFT.

The question is how these findings should affect clinical practice. Firstly, one should realize that the 'solution' of crushed tablets in water is in fact a suspension. The 1.8 mcg/ml of dissolved levothyroxine (in water) that we have found in this study will not be reached in practice, as we have used advanced preparation techniques (ultrasonic bath) that are not available at the bedside. If all undissolved tablet particles from the suspension do reach the patient and are ultimately absorbed, there is no therapeutic problem. However, the data from our study suggest that significant amounts of active drug do not reach the patient when a NFT is

used. Biological availability can be further reduced by interaction of levothyroxine with other substances such as liquid food in the NFT.

Also the administration method as propagated by the Dutch practice guideline (crushed tablets administered with some water on a spoon) raises questions with regard to the reliability of this method, as the majority of the intended dose will be on the bottom of the spoon, and not dissolved in the water administered. One could therefore conclude that preparations available in the Netherlands do not include a form that is suitable for administration to infants and toddlers.

We have shown that these practical issues can be circumvented by using a preparation in which levothyroxine is fully dissolved. A levothyroxine solution was found to be marketed in several European countries. Bio-equivalence between one of these solutions and tablets has been demonstrated in a series of adult patients (4). In a series of 28 neonates with congenital hypothyroidism treated with levothyroxine, parents were more satisfied with this dosage form than parents from a control group in which levothyroxine was prescribed as tablets (5).

Based on this study, the Leiden University Medical Center has acquired the levothyroxine solution through import from Germany, to make it available for young patients. The import of a foreign preparation does create issues in logistics, reimbursement, pharmacovigilance and the availability of a product information leaflet in Dutch.

On the neonatal wards as well as the PICU, we currently prefer use of the 100 mcg/ml (1 drop = 5 mcg). A possible downside tot his product is the fact that it contains propylene glycol as excipient. The FDA recommends a maximum exposure of ≤25 mg/kg/day in prolonged use. The product information does not mention the amount of propylene glycol in the product. The amount of propylene glycol was assessed semi-quantitavely with HPLC, showing that the levothyroxine solution can be safely administered at dosage levels ≤10 mcg/kg/day during longer periods to (preterm) neonates and toddlers, as long as no other preparations with propylene glycol as an excipient are concomitantly administered.

Conclusion

This study shows that us of levothyroxine tablets in young children and patients with a NFT introduces a certain amount of uncertainty caused by poor solubility of levothyroxine in water, and undissolved tablet particles staying behind in the NFT or on the spoon used for administration of crushed tablets. A levothyroxine preparation suitable for use in these populations is not available in the Netherlands.

The issues in levothyroxine administration can be avoided by using a levothyroxine solution available in several other European countries. The authors therefore recommend use of a levothyroxine solution for newborns, infants or patients with a nasogastric feeding tube.

TABLE 1 Assessment of levothyroxine solutions

	Initial levothyroxine concentration (mcg/ml) #	Loss in NFT when compared to initial concentration #
Tablets in water	1.8	22%
Tablets in 4.2% sodium bicarbonate, pH ~ 8	13.1	23%
Reference solution, pH ~ 10	15.0	2%
Commercial preparation	100.0	2%

Values are the averaged result of two paired measurements. The intended initial concentration (with the exception of the commercial preparation of 100 mcg/ml) was 15 mcg/ml. This concentration was not reached due to poor solubility of levothyroxine at pH<10.

1 van Trotsenburg P, Verkerk P, Kempers M, Visser W, van Tijn D, Vulsma T, et al. Werkboek Congenitale Hypothyreoidie. 4th ed. the Netherlands: Paediatric Association of the Netherlands; 2010.
2 KNMP knowledge base, Z-index, http://www.kennisbank.knmp.nl/index.asp.
3 Informatorium Medicamentorum 2010, Geneesmiddel Informatie Centrum, KNMP.
4 Grussendorf M, Vaupel R, Wegscheider K. Bioaquivalenz von L-Thyroxin-Tabletten und L-Thyroxin-Tropfen bei der Behandlung der Hypothyreose in der taglichen Praxis. [Bioequivalence of L-thyroxine tablets and a liquid L-thyroxine solution in the treatment of hypothyroid patients]. Med Klin (Munich). 2004;99(11):639-44. DOI 10.1007/s00063-004-1096-4
5 von Heppe JH, Krude H, L'Allemand D, Schnabel D, Gruters A. The use of L-T4 as liquid solution improves the practicability and individualized dosage in newborns and infants with congenital hypothyroidism. J Pediatr Endocrinol Metab. 2004;17(7):967-74.

CHAPTER 4

Pharmacokinetics and pharmacodynamics of orally administered clonidine – a model-based approach

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Abstract

BACKGROUND The oral clonidine test is a diagnostic procedure performed in children with suspected growth hormone (GH) deficiency. It is associated with untoward effects, including bradycardia, hypotension and sedation. Serum clonidine levels have not previously been assessed during this test.

METHOD In 40 children referred for an oral clonidine test, blood samples were drawn for clonidine and GH. Vital statistics and sedation scores were recorded until 210 minutes post-dose. We explored the relationship between clonidine concentrations and effects such as GH and blood pressure.

RESULTS Of 40 participants, 5 children were GH deficient. Peak clonidine concentrations of 0.846±0.288 ng/mL were reached after one hour. Serum levels declined slowly, with concentrations of 0.701±0.189 ng/mL 210 minutes post-dose. A large inter-individual variation of serum levels was observed. During the procedure systolic BP dropped by 12.8%, diastolic BP by 19.7% and heart rate by 8.4%. Moderate sedation levels were observed. Concentration-effect modeling showed that the amount of GH available for secretion as determined by previous bursts was an important factor influencing GH response.

CONCLUSIONS Clonidine concentrations during the test were higher than necessary according to model-based predictions. A lower clonidine dose may be sufficient, and may produce less side-effects.

Introduction

Children with short stature are frequently referred to a pediatrician or pediatric endocrinologist for assessment of an underlying cause. Short stature may be caused by a variety of conditions, one of which is a growth hormone (GH) deficiency. Several diagnostic procedures are currently employed to assess the adequacy of pituitary GH secretion. Many clinicians use serum IGF-I as a screening parameter, and if serum IGF-I is below the mean for age, one or two GH provocation tests are carried out. These tests are based on the effect on GH secretion of a number of stimuli, including insulin-induced hypoglycemia (insulin-tolerance test, ITT), exercise, sleep (12 or 24 hour GH profile) and a range of pharmacological challenges (centrally acting alfa-receptor agonists, beta receptor antagonists, dopaminergic agonists and serotonergic agonists) (1). Because of the risks of severe hypoglycemia during the ITT (2), in many clinics one of the other pharmacological tests is used, particularly the clonidine test.

Clonidine, a centrally acting alfa2 receptor agonist, was first reported as being a useful tool in assessing the GH axis in the 1970's (3, 4). It was administered in a dose of 0.15 mg per square meter of body surface area (BSA), the dose still used in clinical practice today. Clonidine was demonstrated to elicit a substantial release of GH in healthy controls as compared to patients with hypopituitarism (3).

The diagnostic procedure in suspected GH deficiency currently consists of oral administration of clonidine in a fasted patient, followed by repeated blood samples until 150 minutes post-dose (1). In a non-deficient patient, this generally leads to a significant rise in serum GH levels with a peak usually occurring within 90 minutes after administration of clonidine (5). The diagnosis of GH deficiency will be rejected if GH rises above a pre-defined cut-off during the procedure. Currently, a cut-off level of 6.7 µg/L is used in many centers. The test is associated with several untoward effects (6), including hypotension, bradycardia, moderate sedation and hypoglycemia. At higher dose levels (accidental intoxication),

more serious effects such as coma, respiratory depression and generalized hypotonia have been reported (7). Both the intended (GH release) as unintended effects may well be related to serum levels of clonidine during the test, but this has not been studied so far. In this study we investigated serum levels and pharmacodynamic properties of oral clonidine in children with short stature during the oral clonidine test. To predict the effects of lower dose levels of clonidine, an approach using non-linear mixed effects modeling was employed.

Patients and Methods

40 patients suspected of having GH deficiency and admitted for a clonidine test were included in this study. The study protocol was approved by the Medical Ethics Committee of the Leiden University Medical Center. All study procedures were in accordance with the Declaration of Helsinki. Study participants were recruited from the Leiden University Medical Center (Leiden, the Netherlands), The Juliana Children's Hospital (The Hague, the Netherlands), and the Reinier de Graaf hospital (Delft, the Netherlands). Patients and/or their parents were approached prior to admission, and written informed consent was obtained from parents prior to study participation. All patients with an indication for a clonidine test were eligible for study participation and there were no exclusion criteria.

CLONIDINE ADMINISTRATION

A dose of 150 µg clonidine per square meter body surface area was administered orally after completion of baseline measurements. We used the IV solution (Catapresan®, Boehringer Ingelheim, Germany). In children with a BSA exceeding 1 square meter, no more than 150 µg of clonidine was administered according to the local protocol.

PHARMACOKINETIC SAMPLING AND ANALYSIS

In addition to blood samples taken for GH analysis, a total of 9 samples per patient were drawn from an intravenous cannula for measurement of clonidine levels (before, and at 10, 20, 30, 60, 90, 120, 150 and 210 minutes after clonidine dosing). Serum samples were stored at -40°C until analysis. Clonidine was determined in serum using High Performance Liquid Chromatography (LC 20-A, Shimadzu corporation, Kyoto, Japan), linked to a tandem mass spectrometer (API-4000, Applied Biosystems, Carlsbad CA, USA) at the Clinical Pharmacology Research Center of the Peking Union Medical College Hospital, Beijing, China. The assay had a lower limit of quantitation of 0.1 ng/mL, and the assay was validated using quality control (QC) samples of 0.25 (low), 5 (medium) and 80 (high) ng/mL using codeine as internal standard. The inter-day coefficient of variation was 8.1% for the low QC sample, 2.6% for the medium QC sample and 5.9% for the high QC sample. The intra-day coefficient of variation was 8.3-10.4 % for the low QC sample, 2.3-5.4% for the medium QC sample and 5-11.5% for the high QC sample.

PHARMACODYNAMIC ASSESSMENTS

Vital signs (blood pressure, heart rate, respiratory rate and oxygen saturation) were recorded at 15 minute intervals during the test. Sedation levels were recorded using the modified Ramsay sedation score (MRSS) (8). The definitions of consciousness states of the MRSS are shown in table 1. All assessments in all 40 patients were performed by the same investigator (RK). Serum levels of GH and cortisol were measured in blood samples taken at baseline, and at 10, 20, 30, 60, 90, 120, 150 and 210 minutes after administration of clonidine.

GH was analysed on an Immulite 2500 immunoanalyser (Immuno-LuminoMetric-Assay, ILMA, Siemens Healthcare Diagnostics, Tarrytown (NY), USA). The analytical variation was 5.5%. GH results were expressed

in mass units, in accordance with the most recent consensus statement (IS 98/574; 1 mg equals 3 IU) (9). Cortisol was analysed on a Modular E170 Immunoanalyser (Roche Diagnostics, Mannheim, Germany). The analytical variation was 3.4%. Glucose was analysed using a routine hexokinase method on a Modular P800 analyser (Roche Diagnostics). The analytical variation was 2.5%.

STATISTICS

Paired t-tests were performed to compare the baseline measurements with the mean post-dose measurements and to compare the baseline measurements with the maximum effect values per patient. Analyses of GH, cortisol and serum glucose were performed for two separate groups (GH deficient vs. non-GH deficient). The measurements of the variables GH and cortisol were log-transformed prior to analysis to correct for the expected log-normal distribution of the data. As normal values for blood pressure and heart rate are age dependent, an additional analysis was performed on calculated percentage change from baseline measurements. A one-sample t-test was performed to test if the percentage change from baseline values differed from 0.

A mixed model analysis of variance (with time, sub-group, time*sub-group as fixed factors and subject as random factor) was employed to determine whether presence of GH deficiency influenced the obtained pharmacodynamic measurements. All calculations were performed using SAS for windows V9.1.3 (SAS Institute, Inc., Cary, NC, USA).

The data were analyzed with a non-linear mixed effects modeling approach to explore the relationship between clonidine concentrations and effects using the software package NONMEM v7.2.0 (10). This analysis included modeling of the clonidine pharmacokinetics and the effects of clonidine on GH release, blood pressure and heart rate. The approach entails simultaneous estimation of typical PK and PD model parameters as well as the variability of these parameters within the study population.

Additionally, parameters characterizing individual subjects were derived from the estimated typical values and their variability. With this approach it is possible to plot the effect profiles over time, while the clonidine concentrations are taken into account and determine the shape of the profile.

Results

Characteristics of the study population are shown in table 2. In 33 patients the clonidine test resulted in a GH response above the threshold of 6.7 µg/L, precluding a diagnosis of GH deficiency. Of the remaining 7 patients, a diagnosis of GH deficiency was rejected in 2 patients after normal GH responses during a subsequent arginine test, resulting in 5 patients diagnosed with GH deficiency.

The serum clonidine concentrations increased rapidly with average (± SD) peak concentrations of 0.846 ± 0.288 ng/mL at 1 hour (figure 1). The decline in clonidine serum concentrations was slow, with an average clonidine concentration of 0.701 ± 0.189 ng/mL at 210 minutes post-dose. The data could be well described with a one-compartment model.

Measurements of GH, cortisol and glucose are represented graphically in graphs 2a-c, and summarized in table 3. In non-deficient patients, GH levels increased to a peak around 60 minutes, with an average GH concentration of 15 µg/L at 60 minutes post-dose, and 13 had GH levels exceeding 6.7 µg/L before administration of clonidine. All patients with a GH response >6.7 µg/L had reached this cut-off at or before 90 minutes post-dose. Cortisol levels declined slightly in both deficient and non-deficient patients. Glucose levels did not change significantly during the clonidine test. Three procedures had to be terminated due to hypoglycemia (2.9 mmol/L at 30 minutes in a GH deficient patient aged 4.2 yrs; 2.4 mmol/L at 90 minutes in a GH deficient patient aged 5.1 yrs; and 2.5 mmol/L at 120 minutes in a non-GH deficient patient aged 3.8 yrs

respectively). Mixed model analysis of variance showed that a diagnosis of GH deficiency was associated with a greater maximal decrease in serum glucose (effect size -0.6 mmol/L, 95% CI -1.1 to 0.0, p=0.0571) and minimum serum cortisol (effect size 69.5%, 95% CI -0.4 to 188.6%, p=0.0519).

Cardiovascular measurements for the entire group of patients are represented in graphs 3a-c, and in table 3. After clonidine administration, systolic blood pressure dropped by 12.8% on average in participating patients. Diastolic blood pressure declined by 19.7%. A separate analysis on averaged minimum values per subject showed a decline in systolic blood pressure of 23%, and a decline in diastolic blood pressure of 35.7%. Heart rate declined by 8.4% on average, averaged minimum values were 20.3% below baseline. There were no differences in the cardiovascular measurements between patients with or without GH deficiency.

The observed modified Ramsay Sedation Score (mRSS) scores are represented in figure 4. As expected, all patients had a normal level of consciousness (mRSS 1) at baseline. After clonidine administration, its sedative effects rapidly become apparent, with effects persisting throughout the procedure. The highest levels of sedation were observed between 1 and 2 hours after administration of clonidine, with up to 83% of children having an mRSS of 3 or higher.

The pharmacokinetic model fitted well to the data and could be used to investigate the relationship between the clonidine concentrations and its effects on blood pressure, heart rate and GH. We first explored if there were differences in blood pressure and heart rate between normal and GH deficient children using a mixed model analysis of variance. This showed that there was no influence of presence or absence of a GH deficiency on cardiovascular measurements.

The model to describe the effect of clonidine on GH dynamics was based upon the assumption that it would be released according to a first-order process that was boosted by clonidine. It was further assumed that the GH reserve was finite, and depletion was a limiting factor for further release. Thus, GH release preceding clonidine administration was taken into account. The model described the individual GH-time profiles well for several conditions. Figure 5 shows some typical conditions that may be met during the test. This includes a patient (nr 6) with GH deficiency with low levels before and after clonidine administration. In patient 25 the pre-test GH levels were low, suggesting no previous burst, leading to high GH concentrations after clonidine administration. The model could also adequately capture situations as observed in patient 34 and 35 where GH levels before clonidine administration in subjects were elevated, most likely due to a previous burst. In these cases lower GH peak concentrations after clonidine administration were observed. Simulations based on the model (figure 6) with different doses of clonidine suggest that a substantial GH release (that will allow to confirm or refute the diagnosis of GH deficiency) can be reached with lower doses than the commonly used dose of 150 µg/m^2. The effects of different doses of clonidine on systolic and diastolic blood pressure measurements were also simulated and showed dose dependency (figure 6b).

Discussion

In this study, we collected both pharmacokinetic and pharmacodynamic data during the oral clonidine test. This test is frequently used to investigate the GH axis in children with short stature, to confirm or exclude GH deficiency as an underlying cause. Although frequently used, this procedure is associated with several adverse events, including hypotension, bradycardia and hypoglycemia. We demonstrated a rapid rise in serum clonidine levels after oral administration, with slow clearance. We also observed a significant decline of blood pressure and heart rate, as well as moderate levels of sedation during the test procedure.

Few formal pharmacokinetics studies of clonidine have been performed in children. Published data suggest a terminal half-life of clonidine of 9-12.5 hours (11, 12). This implies that the effects of clonidine can be

expected to be relatively long-lasting. In our study, peak clonidine serum levels were reached at about 60 minutes post-dose and concentrations declined only slowly thereafter. This observation matches previously published data, which showed peak plasma concentrations at 60 minutes post dose (13). The large inter-individual variation can in part be attributed to inter-individual differences in oral bio-availability, which is estimated at 46.9-65.4% (13). It may also be due to the fact that an intravenous formulation was administered orally.

As anticipated, a significant decline in blood pressure and heart rate was observed. These effects are mediated through clonidine's centrally acting alfa2 agonistic effect in a dose-dependent manner. This effect may create a safety issue for patients who undergo this procedure, as in some cases the fall in blood pressure can be clinically relevant. On the other hand, to our knowledge no permanent sequelae have been reported in the literature, arising from clonidine administration in this setting.

The sedative effect of clonidine was demonstrated using the MRSS during the 210 minute observation period in our study. We observed moderate levels of sedation. This is not surprising, as clonidine levels >0.3 ng/mL are viewed as adequate to achieve pre-operative sedation (14). Even at 210 minutes post dose, serum concentrations were 0.7 ng/mL on average. Hypoglycaemia has been associated with the oral clonidine test (15). In our series, 3 procedures had to be terminated due to hypoglycemia, 2 of which in children later confirmed to have a GH deficiency. If decreased serum glucose levels were a direct clonidine effect, one could expect this effect to become apparent in the data presented in figure 2c. As hypoglycemia seems to be a sporadic event during the oral clonidine test, one could hypothesize that individual susceptibility (e.g. GH deficiency) in combination with prolonged fasting for the clonidine test, rather than a generic clonidine effect, is an explanation for hypoglycemia. It has to be kept in mind that the procedure is performed under fasting conditions, so under these circumstances this individual susceptibility might become apparent independent from clonidine administration.

In our study, all of the patients with a GH response >6.7 µg/L during the oral clonidine test had reached this cut-off level at or before 90 minutes post-dose. This finding supports the suggestion by Galluzzi et al (5) to limit the procedure to 90 minutes. On the other hand, one could wonder whether it would be safe to discharge patients at this timepoint, given the persisting effects on blood pressure and sedation.

The exploratory analyses that we performed on the relationship between clonidine concentrations and pharmacodynamics responses (cardiovascular and GH) seem to suggest that the clonidine levels may be higher than necessary. Indeed simulations performed with lower doses suggest that it is possible to select a lower clonidine test dose that is still suitable as GH provocation test while having less effects on blood pressure. In our opinion, this observation should renew interest in a short publication by Laron et al (16), in which a much lower oral dose of 0.025 mg clonidine was shown to be effective in eliciting a substantial release of GH. On the other hand, later studies with clonidine doses of 0.025, 0.050 and 0.1 mg have shown lower peak levels of GH as well as a lower fraction of children reaching the GH cut-off level (17, 18). In this respect our model could be helpful as it will take into account also the GH concentrations before the test and thus putting the GH concentrations after the test in a better perspective. The data suggest that following this approach and taking 3-4 samples for GH before the start of the test would improve the current test's diagnostic sensitivity. This would allow detecting if the patient had a GH peak before the clonidine administration which could influence the response to the test. We also advocate using standardized oral clonidine formulations as it seems likely that the kinetics and the effects depend on the formulation. It may even be considered to use IV administration to reduce the inter-patient variability, but this advantage may be off-set by the expected stronger effects on blood pressure and heart rate.

The safety of the clonidine test might improve by performing a pharmacological intervention during the procedure, thereby stabilizing blood

pressure. For instance, atropine is frequently combined with clonidine as pre-medication in anaesthesiology (19). However, any additional intervention during the clonidine test would have to be scrutinized prior to implementation in practice, as this intervention in its own may have an effect on the GH axis, thereby rendering this combination unsuitable for a clonidine test. For instance, the combination with atropine would completely suppress GH secretion (20). Another intervention of interest could be (oral) fluid loading during the procedure. In a cohort of children who drank 10 ml/kg of diet sprite twice during a combined arginine-clonidine test procedure, less requirement for intravenous fluids and higher blood pressure were observed, with no apparent effect on GH secretion (21).

In conclusion, our study has demonstrated that during the routinely applied clonidine test for the evaluation of GH deficiency high clonidine concentrations were reached. The concentrations were generally higher when compared to target levels used in anesthetic practice. As expected, significant untoward effects were observed, in particular hypotension and sedation. As our results suggest that clonidine concentrations are well above levels required for the intended effect (GH release), we propose a modification of the clonidine challenge with a lower dose. The sampling period of GH could be limited to 90 minutes, as previous literature and our study have demonstrated that maximum GH concentrations have been reached at or before this time point. However, it could be argued that a clinical observation well beyond 90 minutes post-dose is warranted to monitor for clonidine's adverse effects.

TABLE 1 The modified Ramsay sedation score

Score	Definition
1	Awake and alert, minimal or no cognitive impairment
2	Awake but tranquil, purposeful responses to verbal commands at conversation level
3	Appears asleep, purposeful responses to verbal commands at conversation level
4	Appears asleep, purposeful responses to verbal commands but at louder than usual conversation level or requiring light glabellar tap
5	Asleep, sluggish purposeful responses only to loud verbal commands or strong glabellar tap
6	Asleep, sluggish purposeful responses only to painful stimuli
7	Asleep, reflex withdrawal to painful stimuli only (no purposeful response)
8	Unresponsive to external stimuli, including pain

TABLE 2 Study population characteristics*

	GH deficient	Not GH deficient
Age (yrs)	6.00 (± 3.60)	9.30 (± 3.85)
Sex	3 male; 2 female	19 male; 16 female
Height (cm)	107.2 (± 20.7)	123.6 cm (± 20.5 cm)
Height SDS	-2.20 (± 0.31)	-2.46 (± 0.63)
Weight (kg)	19.70 (± 9.39)	25.08 kg (± 9.78 kg)
BMI SDS	0.32 (± 0.78)	-0.65 (± 1.00)

* Mean (± SD)

TABLE 3 Summary of cortisol, serum glucose, growth hormone and vital statistics. Baseline measurements, the difference of mean post-dose measurements compared to baseline and the difference of averaged minimum** measurements for every patient compared to baseline are presented with 95% confidence intervals.

		Cortisol*	Glucose	Growth Hormone*	Systolic BP	Diastolic BP	Heart Rate	Respiratory Rate
Not GH def	Baseline (mean ± SD)	0.347 (±0.23) nmol/L	4.3 (±0.55) mmol/L	2.49 (±4.82) µg/L	104.3 (±9.18) mmHg	56.6 (±6.98) mmHg	85.0 (±16.35) bpm	20.3 (±3.7) /min
	Mean post-dose – Baseline	-45.71 (-64.6 to -29.0) %	0.0 (-0.1 to 0.2) mmol/L	43.03 (19.0 to 59.9) %	-12.3 (-13.9 to -10.7) %	-19.1 (-22.2 to -16.0) %	-9.5 (-12.7 to -6.3) %	-9.7 (-15.6 to -3.9) %
	Min. post-dose – Baseline**	-153.0 (-196.1 to -116.1) %	0.3 (0.1 to 0.5) mmol/L	82.45 (73.0 to 88.6) %	-22.7 (-24.9 to -20.4) %	-35.4 (-38.7 to -32.1) %	-20.4 (-23.6 to -17.1) %	-26.3 (-31.1 to -21.6) %
GH-def	Baseline (mean ± SD)	0.420 (±0.36) nmol/L	3.9 (±0.21) mmol/L	1.94 (±4.66) µg/L	108.0 (±28.36) mmHg	65.8 (±17.91) mmHg	87.4 (±20.38) bpm	24.0 (±1.73) /min
	Mean post-dose – Baseline	-18.58 (-42.1 to 1.0) %	-0.1 (-0.9 to 0.7) mmol/L	0.87 (-162.3 to 61.2) %	-16.3 (-34.2 to 1.5) %	-23.9(-36.5 to -11.2) %	-0.3 (-19.8 to 19.1) %	-14.4 (-60.0 to 31.2) %
	Min. post-dose – Baseline**	-80.6 (-173.8 to -19.2) %	0.2 (-0.6 to 0.9) mmol/L	33.51 (-50.9 to 70.7) %	-25.2 (-45.0 to -5.4) %	-38.2 (-5.7 to -24.7) %	-19.8 (-27.2 to -12.4) %	-37.8 (-76.3 to 0.6) %
All	Baseline (mean ± SD)	0.354 (±0.23) nmol/L	4.3 (±0.54) mmol/L	2.43 (±4.70) µg/L	104.8 (±12.55) mmHg	57.8 (±9.21) mmHg	85.3 (±16.62) bpm	20.6 (±3.71) /min
	Mean post-dose – Baseline	-42.66 (-59.6 to – 27.6) %	0.0 (-0.1 to 0.1) mmol/L	39.59 (16.5 to 56.3) %	-12.8 (-14.9 to -10.7) %	-19.7 (-22.7 to -16.8) %	-8.4 (-11.8 to -5.0) %	-10.1 (-15.7 to -4.6) %
	Min. post-dose – Baseline**	-144.4 (-182.9 to -111.1) %	0.3 (0.1 to 0.4) mmol/L	79.88 (69.7 to 86.6) %	-23.0 (-25.5 to -20.4) %	-35.7 (-38.8 to -32.6) %	-20.3 (-23.2 to -17.4) %	-27.3 (-31.9 to -22.7) %

* Analyses were performed on log-transformed data
** Max. post dose- baseline for Glucose and Growth hormone

FIGURE 1 Serum clonidine concentration-time profile after administration of oral solution of 150 µg clonidine/m² at t=0' in 40 pediatric patients. Individual measurements are represented with dots, the solid line indicates the mean profile based on the pharmacokinetic model that was constructed on the basis of the full dataset. The shaded area represents the 95% prediction interval around the mean.

FIGURE 2A-C GH, cortisol and glucose levels with SD error bars in GH deficient (•) and non-deficient (o) patients during the oral clonidine test.

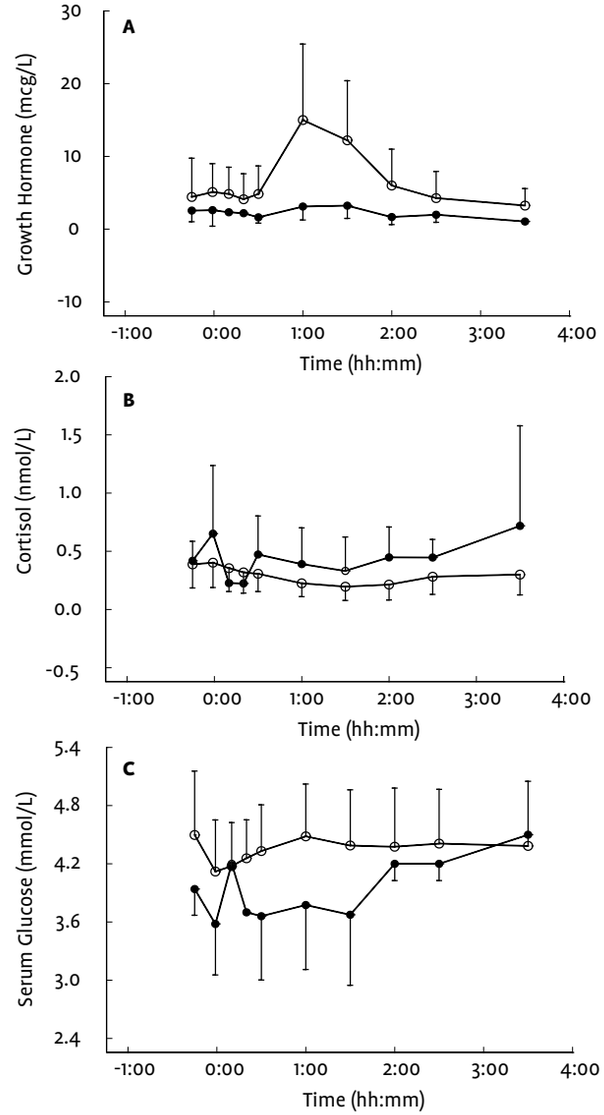


FIGURE 3A-C Blood pressure and heart rate (expressed as % change from baseline, with SD error bars) after 150 µg oral clonidine in children suspected of GH deficiency.

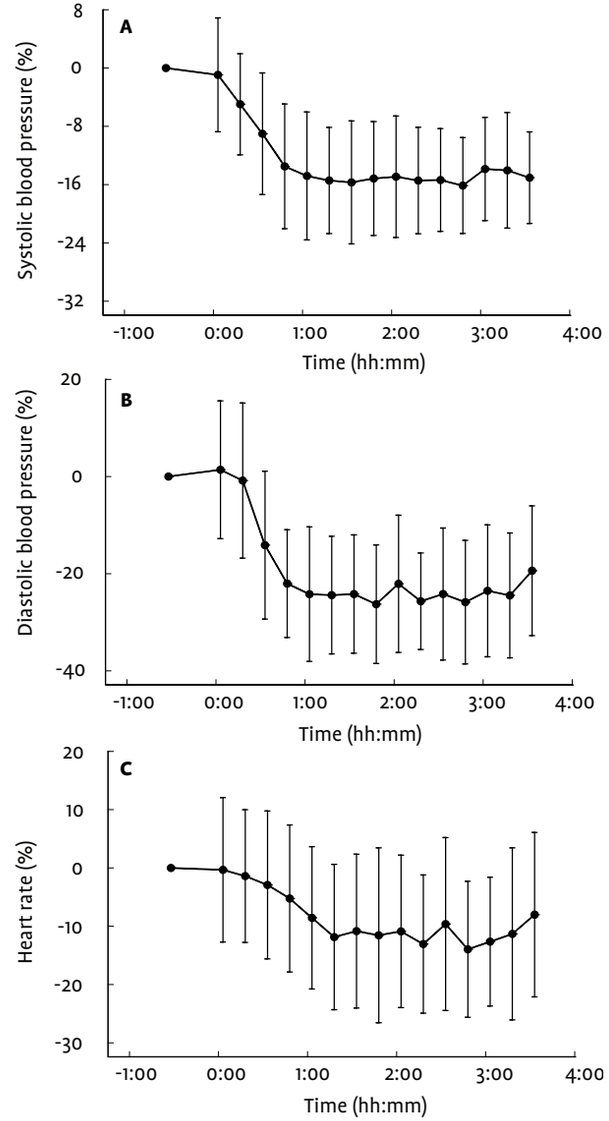


FIGURE 4 Modified Ramsay sedation scores during the oral clonidine test. Data of GH deficient and non-deficient patients were pooled.

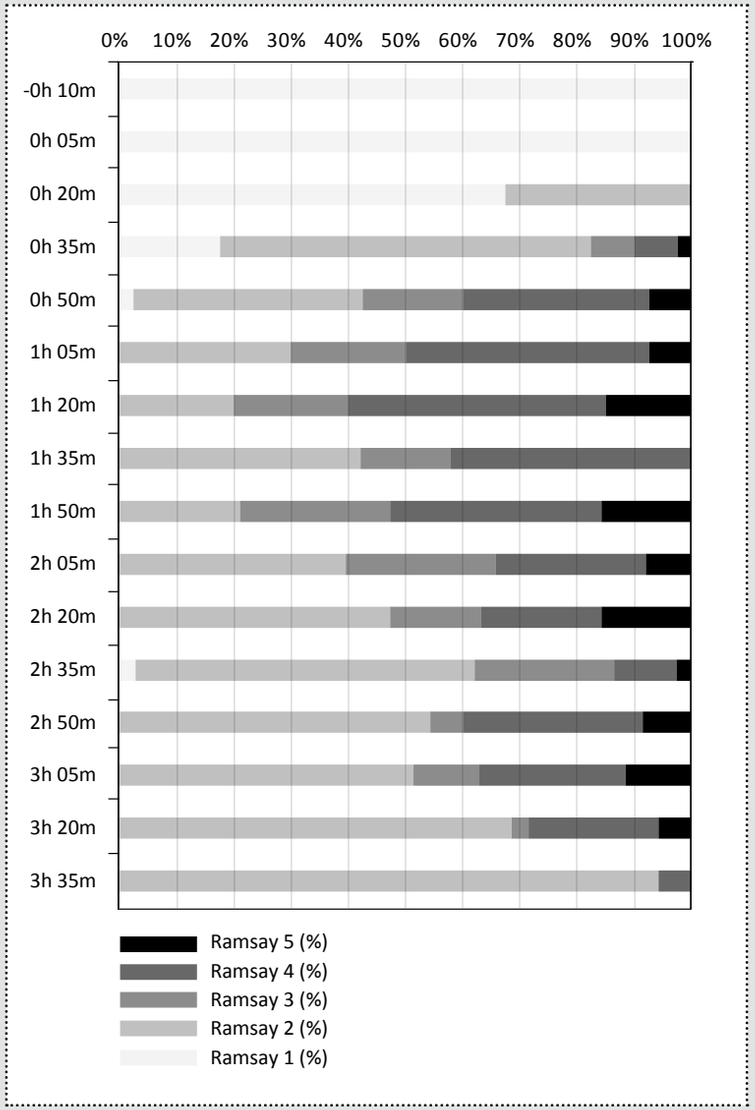


FIGURE 5 Observed GH profiles after clonidine administration for 4 typical patients. Symbols represent actual measurements, the lines show the model-based prediction of the GH response that could be expected taking into account the presence or absence of GH deficiency, as well as absence/presence and the timing of previous GH peaks. Patient 6 (triangle) shows an absent response in GH because of GH deficiency; patient 25 (dots) had low pre-test GH concentrations and the model predicted a robust GH response; patient 34 (rhombus) was supposed to have a peak occurring well before the clonidine administration and was expected to have a minimal increase in GH after clonidine and patient 35 (squares) may have had a robust GH peak just before the clonidine administration so that clonidine had only a minimal effect.

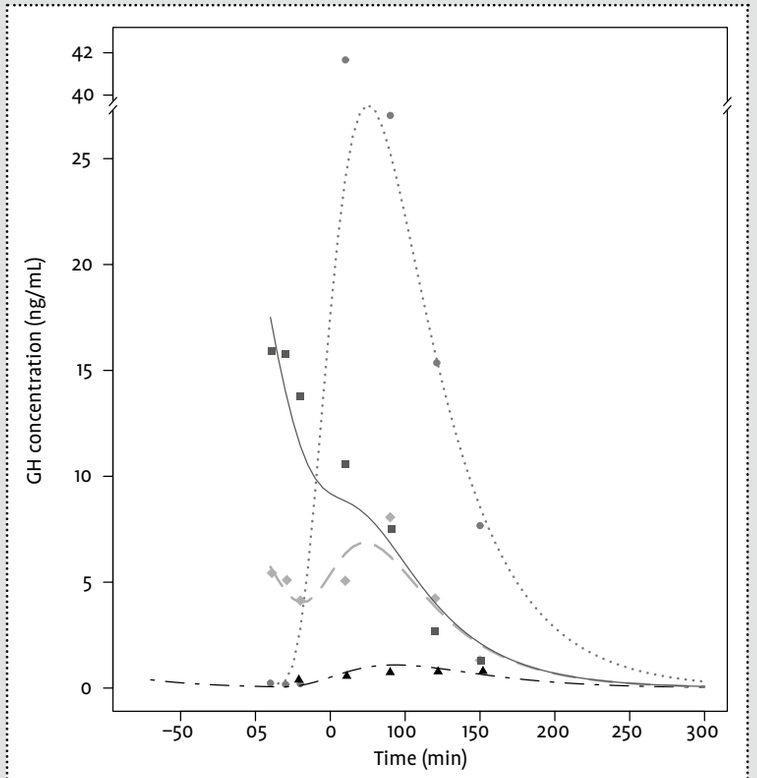
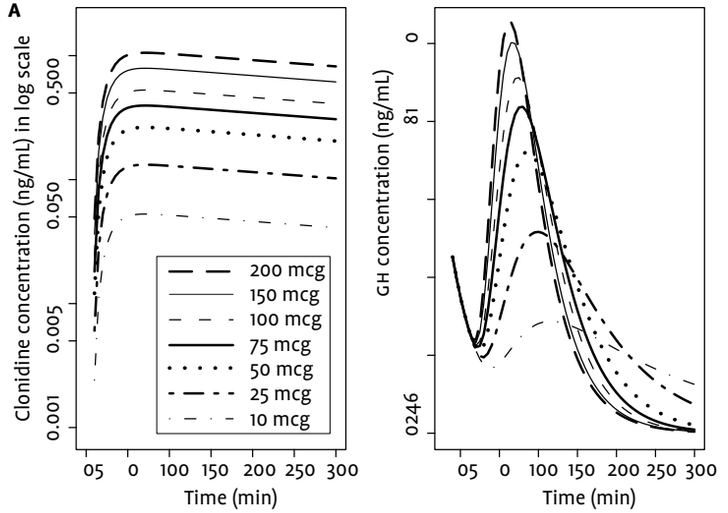
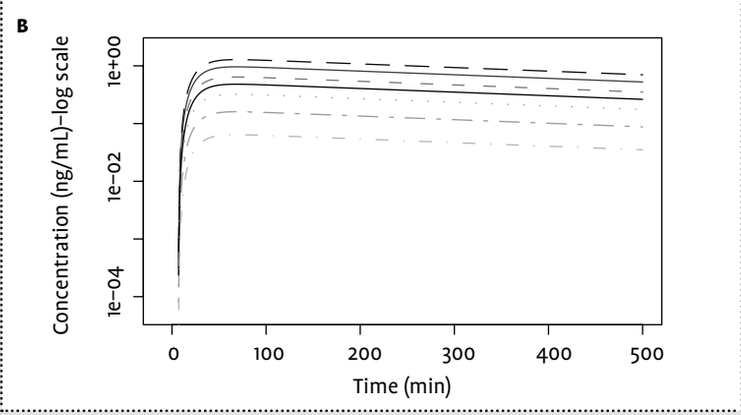
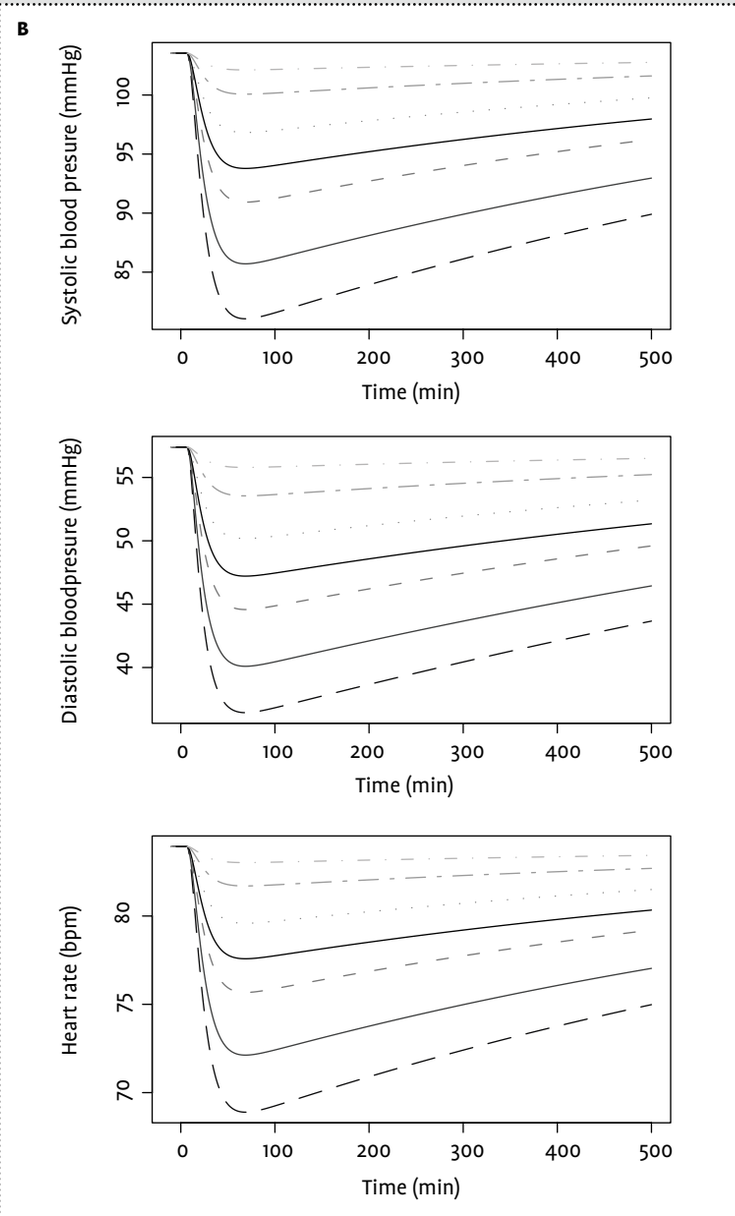


FIGURE 6A-B Simulations of GH profiles (a) and effects on blood pressure and heart rate (b) in a typical pediatric population using different doses of clonidine. Clonidine doses ranging from 200 to 10 µg (200, 150, 100, 75, 50, 25 and 10 µg) were used to simulate the response to the test. In these simulations, a body surface area of 0.903 m² was assumed (average of the study population).







1 Ranke MB. Growth Hormone Deficiency: Diagnostic Principles and Practice. In: Ranke MB, Mullis PE, editors. Diagnostics of Endocrine Function in Children and Adolescents. Basel: Karger; 2011. p. 102-37.

2 Shah A, Stanhope R, Matthew D. Hazards of pharmacological tests of growth hormone secretion in childhood. BMJ. 1992;304(6820):173-4.

3 Gil-Ad I, Topper E, Laron Z. Oral clonidine as a growth hormone stimulation test. Lancet. 1979;2(8137):278-9.

4 Lal S, Tolis G, Martin SB, Brown GM, Guyda H. Effect of clonidine on growth hormone, prolactin, luteinizing hormone, follicle-stimulating hormone, and thyroid-stimulating hormone in the serum of normal men. J Clin Endocrinol Metab. 1975;41(5):827-32.

5 Galluzzi F, Stagi S, Parpagnoli M, Losi S, Pagnini I, Favelli F, et al. Oral clonidine provocative test in the diagnosis of growth hormone deficiency in childhood: should we make the timing uniform? Horm Res. 2006;66(6):285-8. DOI 10.1159/000095781

6 Rachmiel M, Johnson T, Daneman D. Clonidine ingestion in children is not uneventful. J Pediatr. 2006;148(6):850; author reply -1. DOI 10.1016/j.jpeds.2005.12.030

7 Spiller HA, Klein-Schwartz W, Colvin JM, Villalobos D, Johnson PB, Anderson DL. Toxic clonidine ingestion in children. J Pediatr. 2005;146(2):263-6. DOI 10.1016/j.jpeds.2004.09.027

8 Agrawal D, Feldman HA, Krauss B, Waltzman ML. Bispectral index monitoring quantifies depth of sedation during emergency department procedural sedation and analgesia in children. Ann Emerg Med. 2004;43(2):247-55. DOI 10.1016/S0196064403007212

9 Clemmons DR. Consensus statement on the standardization and evaluation of growth hormone and insulin-like growth factor assays. Clin Chem. 2011;57(4):555-9. DOI 10.1373/clinchem.2010.150631

10 Beal S, Sheiner LB, Boeckmann A, Bauer RJ. NONMEM's User's Guides (1989-2009). Ellicott City, MD, USA: Icon Development Solutions; 2009.

11 Lonnqvist PA, Bergendahl HT, Eksborg S. Pharmacokinetics of clonidine after rectal administration in children. Anesthesiology. 1994;81(5):1097-101.

12 Potts AL, Larsson P, Eksborg S, Warman G, Lonnqvist PA, Anderson BJ. Clonidine disposition in children; a population analysis. Paediatr Anaesth. 2007;17(10):924-33. DOI 10.1111/j.1460-9592.2007.02251.x

13 Larsson P, Nordlinder A, Bergendahl HT, Lonnqvist PA, Eksborg S, Almenrader N, et al. Oral bioavailability of clonidine in children. Paediatr Anaesth. 2011;21(3):335-40. DOI 10.1111/j.1460-9592.2010.03397.x

14 Sumiya K, Homma M, Watanabe M, Baba Y, Inomata S, Kihara S, et al. Sedation and plasma concentration of clonidine hydrochloride for pre-anesthetic medication in pediatric surgery. Biol Pharm Bull. 2003;26(4):421-3.

15 Huang C, Banerjee K, Sochett E, Perlman K, Wherrett D, Daneman D. Hypoglycemia associated with clonidine testing for growth hormone deficiency. J Pediatr. 2001;139(2):323-4. DOI 10.1067/mpd.2001.116276

16 Laron Z, Gil-Ad I, Topper E, Kaufman H, Josefsberg Z. Low oral dose of clonidine: an effective screening test for growth hormone deficiency. Acta Paediatr Scand. 1982;71(5):847-8.

17 Lanes R, Recker B, Fort P, Lifshitz F. Low-dose oral clonidine. A simple and reliable growth hormone screening test for children. Am J Dis Child. 1985;139(1):87-8.

18 Menon PS, Gupta P, Karmarkar MG. High and low dose clonidine tests for the diagnosis of growth hormone deficiency. Indian Pediatr. 1994;31(2):145-51.

19 Bergendahl H, Lonnqvist PA, Eksborg S. Clonidine: an alternative to benzodiazepines for premedication in children. Curr Opin Anaesthesiol. 2005;18(6):608-13. DOI 10.1097/01.aco.0000191891.44314.36

20 Casanueva FF, Villanueva L, Cabranes JA, Cabezas-Cerrato J, Fernandez-Cruz A. Cholinergic mediation of growth hormone secretion elicited by arginine, clonidine, and physical exercise in man. J Clin Endocrinol Metab. 1984;59(3):526-30.

21 May M, Rose SR. Oral hydration during growth hormone stimulation with clonidine. J Pediatr Nurs. 2007;22(5):383-7. DOI 10.1016/j.pedn.2007.01.007

CHAPTER 5

Repeated administration of a neurocognitive test battery in healthy children

Submitted for publication

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Abstract

BACKGROUND This study was performed to assess the suitability of a selection of tasks from a neurocognitive test battery (Neurocart) in healthy children aged 8-12 years. Utilizing the Neurocart in the pediatric age group would be particularly attractive, as it provides a non-invasive method of gathering an extensive amount of data in different settings, including interventional research.

METHODS We designed this study to establish whether it would be feasible to repeatedly complete the neurocognitive tasks and to establish inter- and intra-individual variance of task results upon test repetition. We also assessed the influence of age on the obtained measurements. A short questionnaire was completed upon study completion to evaluate how children had experienced the study procedures.

RESULTS The 15 participating children completed 3 consecutive runs of tasks (Stroop task, body sway, adaptive tracking, smooth pursuit eye movements, saccadic eye movements, finger tapping). A significant learning effect was observed in the Stroop task, smooth pursuit eye movement task, saccadic eye movement tasks and adaptive tracking task. By linear regression analysis, significant effects of age on the Stroop task and body sway task were demonstrated. Judging by the questionnaire results, performing these tasks does not seem to be burdensome for participating children.

CONCLUSION The selection of neurocognitive tasks used in this study seems suitable for interventional research in individuals within the age range of 8-12 years.

Introduction

Neurocognitive tasks can form a useful tool in the research area of clinical pharmacology. Task results can be used to characterize certain study populations, or, more interestingly, can serve as a tool to measure or quantify the (side-) effect of interventions such as the administration of neurotropic drugs. The Neurocart is a test battery consisting of a set of well-known neurocognitive tasks, which has been used extensively in our centre, and which is strongly founded in the literature (1, 2).

Utilizing the Neurocart in the pediatric age group would be particularly attractive, as it provides a non-invasive method of gathering an extensive amount of data in different settings, including (drug) trials. Minimal risk and burden are a requirement for non-therapeutic drug trials in this age group (International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use. ICH Harmonised tripartite guideline E11-Clinical investigation of medicinal products in the pediatric population, 2000). The need for adequately validated and non-invasive biomarkers of drug effects has increased over the past years, with a legal framework in place forcing industry to evaluate new drugs in all age groups (3).

When designing trials to be performed in the pediatric age group, the investigator is faced by some unique challenges. As mentioned earlier, there is a much lower threshold for risk and burden. Secondly, if active participation from the part of a participating child is required, as for instance with neurocognitive tasks, the investigator will have to establish whether children will be able to complete the required tasks without losing their attention. Finally, results may be influenced by developmental differences across age groups. For example, eye movement tasks were shown to be influenced by developmental changes from childhood into adolescence (4, 5).

As a preparatory effort towards utilizing our Neurocart test battery in clinical studies in the pediatric population, we designed a pilot study in

healthy children to establish whether it would be feasible to (repeatedly) complete a set of neurocognitive tasks and to establish inter- and intra-individual variance of task results upon test repetition. We wanted to assess how children experienced participation by means of a short structured questionnaire to confirm our view that these procedures fall within the ethical requirement of 'minimal risk and burden'. Finally, we aimed to assess a possible influence of age on the obtained measurements.

Materials and methods

STUDY DESIGN

This was an observational study in healthy children aged 8-12 years. Exclusion criteria were: any known psychiatric diagnosis (e.g. autism, oppositional defiant disorder, ADHD); dyslexia; learning disability; significant behavioural problems; use of any medication and preterm birth. The Medical Ethics Committee of the Leiden University Medical Centre approved the study protocol. In compliance with the Declaration of Helsinki, written informed consent was obtained from the parents or legal guardians of the participating children.

NEUROCOGNITIVE TASKS

Two single-trial computerized versions of the classic *colour-word Stroop tasks* (6) were presented to the test subjects. In the first trial, 20 coloured items were presented at random. The subjects were asked to respond as fast and as accurate as possible by pressing the keys 1, 2 or 3 on the numerical pad with the index finger, middle finger and ring finger of the dominant hand, corresponding with the correct answer. In the second trial, which appeared directly after the first trial, 20 colour and word pairs were presented randomly to the subject, forming either congruent or incongruent matches. The subjects were again asked to respond as fast as possible by pressing the keys 1, 2 or 3 on the numerical pad, corresponding with the correct answer. In both trials, reaction times and the number of correct responses were recorded.

The *adaptive tracking test* is a pursuit-tracking task. A circle of known dimensions moves randomly about a screen. The test subject must try to keep a dot inside the moving circle by operating a joystick. If this effort is successful, the speed of the moving circle increases. Conversely, the velocity is reduced if the test subject cannot maintain the dot inside the circle. In contrast to non-adaptive tracking methods, this leads to a constant and individually adapted challenge throughout the procedure. Performance is scored after a fixed period. The adaptive tracking test was performed as originally described by Borland and Nicholson (7, 8), using customised equipment and software (Hobbs, 2004, Hertfordshire, UK). The average performance and the standard deviation of scores over a 3.5-minute period were used for analysis. This 3.5-minute period included a run-in time of 0.5 minute; in this run-in time the data were not recorded.

The use of a computer for measurement of *saccadic eye movements* was originally described by Baloh (9). In this study we used the nystagmo stimulator for stimulus display from Nihon Kohden (Nihon Kohden Corporation, Tokyo, Japan), the program for signal collection and the AD-converter from Cambridge Electronic Design (CED Ltd., Cambridge, UK), the amplification by Grass (Grass-Telefactor, An Astro-Med, Inc. Product Group, Braintree, USA) and the sampling and analysis scripts were developed at the CHDR (Leiden, the Netherlands). Disposable electrodes were applied on the forehead and beside the lateral canthi of both eyes of the subject for registration of the electro-oculographic signals. Head movements were minimised with the aid of a head support placed opposite the target. The target consists of an array of light emitting diodes on a bar, fixed at 50 cm in front of the head support. Saccadic eye movements were recorded for stimulus amplitudes of approximately 15 degrees to either side. Fifteen saccades were recorded with interstimulus intervals varying randomly between 3 and 6 seconds. Average values of latency (reaction time), saccadic peak velocity of all *correct*

saccades and inaccuracy of all saccades were used as parameters. Saccadic inaccuracy was calculated as the absolute value of the difference between the stimulus angle and the corresponding saccade, expressed as a percentage of the stimulus angle.

The same system as used for saccadic eye movements was also used for measurement of *smooth pursuit*. For smooth pursuit eye movements, the target moves sinusoidally at frequencies ranging from 0.3 to 1.1 Hz, by steps of 0.1 Hz. The amplitude of target displacement corresponds to 22.5 degrees eyeball rotation to both sides. Four cycles were recorded for each stimulus frequency. The time in which the eyes are in smooth pursuit of the target were calculated for each frequency and expressed as a percentage of stimulus duration. The average percentage of smooth pursuit for all stimulus frequencies was used as parameter.

Finger tapping has been adapted from the Halstead Reitan Test Battery (10), and evaluates motor activation and fluency. In this test, speed of finger tapping was measured for the index finger of the dominant hand; a session contains five 10-second trials. Feedback on performance was given by a counter in the centre of the screen, while the amount of taps of each 10-second trial was shown on the screen in between the trials. The space bar was used as tapping device. The children were instructed to tap as quickly as possible with the index finger and to rest the wrist on the table. The mean tapping rate and the standard deviations for the dominant hand were used for statistical analysis.

The *body sway* meter allows measurement of body movements in a single plane, providing a measure of postural stability. Body sway is measured with an apparatus similar to the Wright ataxiameter (11). With a string attached to the waist, all body movements in the sagittal (forward/backward) plane over a period of 2 minutes are integrated and expressed as mm sway on a digital display. Children were instructed to keep the eyes closed to eliminate the contribution of vision to postural control. Before starting a measurement, children were asked to stand still and comfortable, with their feet approximately 10 cm part and their hands in a relaxed position alongside the body.

STUDY DAYS

Participants were asked to come to the study centre accompanied by their parents for a single occasion, where they consecutively performed three runs of the aforementioned neurocognitive tasks. After completing the three runs of testing, children were asked to complete a structured questionnaire on how they experienced participation, in order to evaluate the research burden.

STATISTICS

To estimate the variances an analysis of variance was done. Body Sway data were log-transformed to meet the requirements for the analysis of variance parameters.

The parameters were analysed with a mixed model analysis of variance with time as fixed factor, subject as random factor and a variance components covariance structure. The covariance parameter estimate for subject is the inter-subject variance, the covariance parameter estimate for the residual the intra-subject variance. To eliminate the age effect from the inter-subject variability, the analysis was repeated with the same model and age as covariate. Coefficients of variation were calculated. Run-to-run contrasts were calculated within the model with estimated means of the difference and 95% confidence intervals. To establish the effect of age on the results of the different measurements, a linear regression analysis was performed using averaged measurements for each test parameter per subject. R square values were calculated, and the null hypothesis of the slope being equal to zero was tested. All statistical calculations were performed using SAS version 9.1.3 (SAS Institute, Inc, Cary, NC, USA).

Results

The study was completed by 15 children (8 males, 7 females; age 10.64 ± 1.31 years).

Means of all measurements (i.e. all measurements for each task pooled), with calculated inter- and intra-individual coefficients of variation are presented in table 1. Estimated means of measurements per run, including run-to-run comparisons are presented in table 2. Run-to-run increase in performance was statistically significant in the Stroop task, Smooth pursuit eye movement, Saccadic eye movements and adaptive tracking tasks.

The linear regression analysis results are presented in table 3. Clear age dependent changes in test results are observed in several neurocognitive tasks, as represented in figures 1a-c. Generally, task performance improves with increasing age. The age dependent change is statistically significant for the Stroop task reaction times and for the body sway task. However, a considerable residual error remains, as indicated by relatively low R-square values.

Results of the post-study questionnaire are presented in table 4. Children tended to rate participation rather positively, with 53.3% of children enjoying participation 'quite much' and 46.6 % of children 'very much'. Two-thirds would participate again when asked. Some tests were clearly appreciated more (tapping task) than others (adaptive tracking).

Discussion

In this study, a battery of neurocognitive tasks (Neurocart) was administered repeatedly in healthy children aged 8-12. For several tasks, repeated administration was shown to increase task performance. Age dependent changes in task performance were also demonstrated. Intra- and inter-individual coefficients of variation, calculated for pooled results of all 3 measurements in all 15 subjects, were relatively large. In part, this observation can be explained by the effects of repeated administration (such as learning effects, boredom, fatigue; leading to increased intra-individual variance), and the effect of age (leading to increased interindividual variance).

Age effects were not observed in any task. Eye movements in particular were strikingly constant within the age range of our study population, suggesting that the neuronal network responsible for eye movements has matured at a young age (12), or that the maturational process is slow within this age range. On the other hand, some tasks displayed clear effects of age. As an example, in our study postural stability improved significantly with increasing age, consistent with existing literature (13). The participating children in our study were all able to complete the tasks. Participation in the study was well tolerated, as reflected by the favorable responses to our post-study questionnaire. In our opinion, performing these tasks can therefore be viewed as 'minimal burden' in the context of the medical-ethical review process.

Learning effects were clearly observed in several of the administered neurocognitive tasks. Other tasks, in particular the simple motor tasks (tapping, eye movements, postural stability) did not show any learning effect). It could be speculated that tasks requiring higher cortical levels display greater learning effects than tasks addressing simple motor abilities.

The main goal of this study was to establish the feasibility of employing the Neurocart in pharmacological intervention studies with children. Judging by the responses to the questionnaire completed after participation, performing the tasks does not seem to be burdensome in this age group. Learning effects and age effects are both factors that would need to be taken into account when employing the Neurocart in interventional research. Studies employing the Neurocart in the pediatric age group should be carefully designed to avoid any confounding by the aforementioned effects. For example, learning effects might be controlled

by performing one or several 'run-in' cycles of testing,and comparison of measurements between different groups would require meticulous age-matching.

In conclusion, the selection of neurocognitive tasks used in this study seems suitable for employing in interventional research in individuals within the age range of 8-12 years. The non-invasive nature of these methods make them particularly suitable for use in pediatric age groups. In the setting of pharmacological research, this would typically entail comparison of task results after administration of active drug or placebo to demonstrate central (side)- effects of the drug studied, and/or repeated administration of tasks to estimate the duration of the observed effect.

TABLE 1 Mean of all obtained measurements (3 measurements per individual), with calculated intra- and inter-subject coefficient of variation and inter-subject coefficient of variation corrected for age.

Parameter	Mean	Intra-subject cv	Inter-subject cv	Inter-subject cv (corrected for age)
Stroop Basic, # correct	18.8	5.12	5.63	5.39
Stroop Basic, reaction time (ms)	681	13.1	19.0	17.1
Stroop Conflict, # correct	19.0	4.97	4.55	4.56
Stroop Conflict, reaction time (ms)	840	13.9	23.3	20.8
Smooth pursuit (%)	34.8	14.0	22.9	23.6
Saccadic Inaccuracy (%)	6.18	23.5	21.7	21.4
Saccadic peak velocity (degr/sec)	537	3.83	8.99	9.33
Saccadic reaction time (ms)	221	9.94	10.1	9.95
Tapping (/10 sec)	55	3.16	11.3	10.3
Tapping St. Dev.	3.84	27.9	25.1	23.6
Adaptive tracking (%)	16.4	11.0	24.3	22.6
Adaptive Tracking St. Dev.	2.78	20.2	13.0	13.4
Body Sway (mm; log-transformed)	5.88	18.0	50.3	40.8

TABLE 2 Least square means estimates for each task per run, and statistical significance of run-to-run contrasts.

Parameter		Est. mean (95% CI)	x Run 2 (p-value)	x Run 3 (p-value)
Stroop Basic, # correct	Run 1	18.7 (18.0-19.4)	0.26	0.85
	Run 2	19.1 (18.4-19.8)	-	0.35
	Run 3	18.7 (18.0-19.4)	-	-
Stroop Basic, reaction time (ms)	Run 1	731 (652-811)	0.06	0.01
	Run 2	669 (589-748)	-	0.43
	Run 3	643 (563-722)	-	-
Stroop Conflict, # correct	Run 1	18.7 (18.1-19.3)	0.70	0.06
	Run 2	18.9 (18.3-19.5)	-	0.13
	Run 3	19.4 (18.8-20.0)	-	-
Stroop Conflict, reaction time (ms)	Run 1	882 (765-999)	0.37	0.06
	Run 2	843 (726-960)	-	0.29
	Run 3	797 (680-914)	-	-
Smooth pursuit (%)	Run 1	31.5 (26.7-36.2)	<0.01	<0.01
	Run 2	36.6 (31.7-41.5)	-	0.83
	Run 3	37.0 (32.1-41.9)	-	-
Saccadic Inaccuracy (%)	Run 1	7.05 (6.08-8.02)	0.10	<0.01
	Run 2	6.09 (5.12-7.06)	-	0.24
	Run 3	5.45 (4.51-6.39)	-	-
Saccadic peak velocity (degr/sec)	Run 1	558 (530-586)	<0.01	<0.0001
	Run 2	531 (503-559)	-	0.21
	Run 3	521 (493-549)	-	-
Saccadic reaction time (ms)	Run 1	214 (199-230)	0.41	0.11
	Run 2	221 (206-237)	-	0.44
	Run 3	228 (213-243)	-	-
Tapping (/10 sec)	Run 1	55.3 (51.8-58.8)	0.28	0.45
	Run 2	54.6 (51.1-58.1)	-	0.76
	Run 3	54.8 (51.3-58.3)	-	-
Tapping St. Dev.	Run 1	3.73 (3.05-4.41)	0.77	0.31
	Run 2	3.61 (2.93-4.30)	-	0.20
	Run 3	4.14 (3.44-4.85)	-	-
Adaptive tracking (%)	Run 1	14.9 (12.6-17.2)	<0.01	<0.01
	Run 2	17.0 (14.7-19.4)	-	0.76
	Run 3	17.3 (14.9-19.6)	-	-
Adaptive Tracking St. Dev.	Run 1	2.75 (2.45-3.05)	0.93	0.66
	Run 2	2.73 (2.43-3.04)	-	0.61
	Run 3	2.84 (2.54-3.14)	-	-
Body Sway (mm; log-transformed)	Run 1	5.84 (5.56-6.12)	0.64	0.17
	Run 2	5.87 (5.59-6.15)	-	0.36
	Run 3	5.93 (5.65-6.22)	-	-

TABLE 3 Tabulated results of linear regression analysis. For each test parameter, measurement results were averaged per subject. The y-axis intercept, regression coefficient (slope of the regression line), R-square values and p-values are presented.

Parameter	y-axis Intercept	Regression coefficient	R-square	p-value (slope ≠ 0)
Stroop Basic, # correct	16.4	0.24	0.11	0.22
Stroop Basic, reaction time (ms)	1194	-50.6	0.30	0.03
Stroop Conflict, # correct	18.9	0.02	0.00	0.88
Stroop Conflict, reaction time (ms)	1660	-79.0	0.29	0.04
Smooth pursuit (%)	44.5	-0.91	0.02	0.59
Saccadic Inaccuracy (%)	9.65	-0.33	0.11	0.24
Saccadic peak velocity (degr/sec)	549	-1.20	0.00	0.91
Saccadic reaction time (ms)	273	-4.90	0.08	0.31
Tapping (/10 sec)	30.1	2.33	0.24	0.06
Tapping St. Dev.	0.06	0.35	0.21	0.08
Adaptive tracking (%)	2.26	1.33	0.19	0.10
Adaptive Tracking St. Dev.	2.52	0.02	0.01	0.75
Body Sway (mm; log-transformed)	8.4	-0.24	0.46	<0.01

TABLE 4 Results of the post-study questionnaires taken after completion of the 3 test-runs.

Question	Answer	(%)
How did you like participating in this study?	Not at all	0
	Not much	0
	Quite much	53.3
	Very much	46.7
How did you like the duration of the tests?	Much too long	0
	Long	33.3
	Not too long	60
	Short	6.7
	Much too short	0
Would you participate again?	Yes	66.7
	No	6.7
	Not sure	26.7
The electrodes on my forehead were:	Very annoying	0
	A little annoying	33.3
	Not so annoying	20
	Not at all annoying	46.7
The task I enjoyed the most was:	Stroop	13.3
	Adaptive tracking	0
	Eye pursuit tasks	33.3
	Body Sway	6.7
	Tapping	46.7
The task I disliked most was:	Stroop	0
	Adaptive tracking	83.3
	Eye pursuit tasks	3.3
	Body Sway	13.3
	Tapping	0

FIG. 1A-C Graphical representation of the linear regression analysis for Stroop reaction time (basic condition), Stroop reaction time (conflict condition) and body sway with age. The dots represent the average of 3 test runs for each subject. (for regression coefficients and R-square values, refer to table 3).

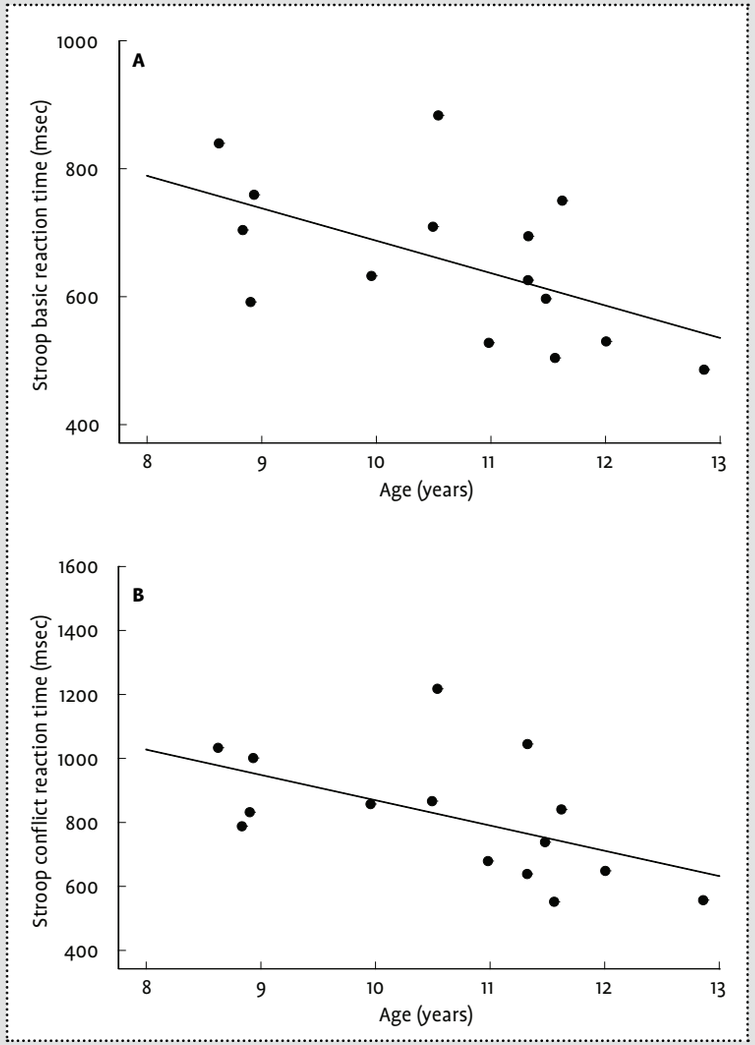
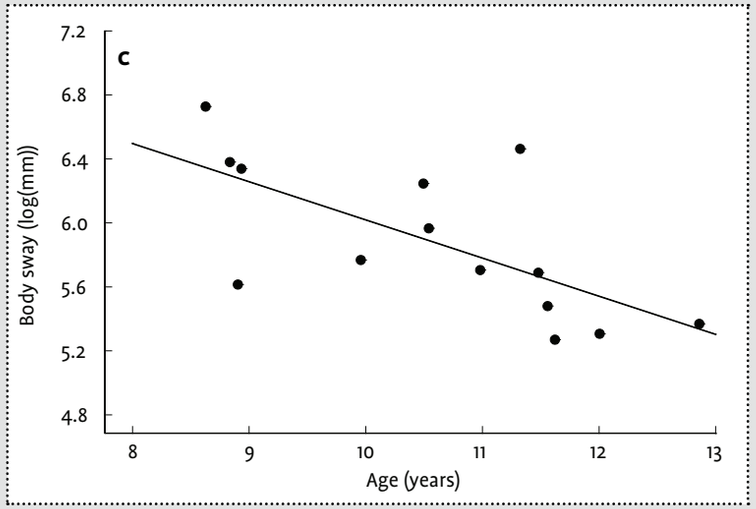


FIG. 1C



1 de Visser SJ, van der Post J, Pieters MS, Cohen AF, van Gerven JM. Biomarkers for the effects of antipsychotic drugs in healthy volunteers. Br J Clin Pharmacol. 2001;51(2):119-32.

2 Dumont GJ, de Visser SJ, Cohen AF, van Gerven JM. Biomarkers for the effects of selective serotonin reuptake inhibitors (SSRIs) in healthy subjects. Br J Clin Pharmacol. 2005;59(5):495-510. DOI 10.1111/j.1365-2125.2005.02342.x

3 Choonara I. Regulation of drugs for children in Europe. BMJ. 2007;335(7632):1221-2. DOI 10.1136/bmj.39400.376424.BE

4 Ross RG, Radant AD, Young DA, Hommer DW. Saccadic eye movements in normal children from 8 to 15 years of age: a developmental study of visuospatial attention. J Autism Dev Disord. 1994;24(4):413-31.

5 Salman MS, Sharpe JA, Lillakas L, Dennis M, Steinbach MJ. Smooth pursuit eye movements in children. Exp Brain Res. 2006;169(1):139-43. DOI 10.1007/s00221-005-0292-7

6 Stroop JR. Studies of interference in serial verbal reactions. Journal of Experimental Psychology. 1935;18:643-62.

7 Borland RG, Nicholson AN. Comparison of the residual effects of two benzodiazepines (nitrazepam and flurazepam hydrochloride) and pentobarbitone sodium on human performance. Br J Clin Pharmacol. 1975;2(1):9-17.

8 Borland RG, Nicholson AN. Visual motor co-ordination and dynamic visual acuity. Br J Clin Pharmacol. 1984;18 Suppl 1:69S-72S.

9 Baloh RW, Sills AW, Kumley WE, Honrubia V. Quantitative measurement of saccade amplitude, duration, and velocity. Neurology. 1975;25(11):1065-70.

10 Andrew JM. Delinquents and the Tapping Test. J Clin Psychol. 1977;33(3):786-91.

11 Wright BM. A simple mechanical ataxia-meter. J Physiol. 1971;218 Suppl:27P-8P.

12 Pieh C, Proudlock F, Gottlob I. Smooth pursuit in infants: maturation and the influence of stimulation. Br J Ophthalmol. 2012;96(1):73-7. DOI 10.1136/bjo.2010.191726

13 Mickle KJ, Munro BJ, Steele JR. Gender and age affect balance performance in primary school-aged children. J Sci Med Sport. 2011;14(3):243-8. DOI 10.1016/j.jsams.2010.11.002

CHAPTER 6

Acute effects of methylphenidate in children with ADHD on a neurocognitive test battery: a randomized, placebo controlled trial

Submitted for publication

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Abstract

RATIONALE The use of neurocognitive tasks as biomarker of drug effects increases the efficiency of pharmacological research. The non-invasive nature of such tasks makes them particularly attractive for application in the pediatric population, as risks and burden of scientific research in this group should be minimal.

OBJECTIVES To establish whether acute effects of methylphenidate can be measured with a neurocognitive test battery (Neurocart) in children with attention deficit hyperactivity disorder (ADHD), to establish how performing these tasks is tolerated by these children, to measure acute cardiovascular effects of methylphenidate, and to measure methylphenidate concentrations in saliva.

METHODS We performed a randomized, placebo-controlled, 2-way cross-over study with 20 children aged 8-12 years with a confirmed diagnosis of ADHD. Data were collected on two separate study days after administration of either methylphenidate or placebo.

RESULTS Acute effects of methylphenidate could be demonstrated in five of the tasks from the Neurocart test battery (body sway, saccadic reaction time, smooth pursuit, finger tapping task and adaptive tracking task). The study was well-tolerated by participating children. A significant rise in heart rate (4/min) and blood pressure (4.5 mmHg systolic, 3.1 mmHg diastolic) was observed 60-120 minutes after methylphenidate administration. Saliva analyses showed maximum methylphenidate concentrations of 25.23 (±22.67) ng/mL at 120 minutes post-dose, with an apparent elimination half-life of approximately 120 minutes.

CONCLUSIONS Acute neurocognitive and cardiovascular effects of methylphenidate can be measured with the Neurocart test battery. This study also demonstrates the feasibility of performing data-intensive studies in this age group with little burden or risk to participants.

Introduction

In the research field of clinical pharmacology, biomarkers are frequently utilized to study drug effects. The use of biomarkers is of increasing importance in the field of drug evaluation and drug development, as the efficiency of research can be increased, and data generated by studies employing biomarkers can be used in PK/PD modeling as well as bridging studies. One form of such biomarkers is the result of neurocognitive tasks in subjects before and after administration of drugs with known or suspected central nervous system (CNS) effects. The Neurocart is a test battery consisting of a set of well-known neurocognitive tasks, with extensive validation in virtually all classes of psychotropic drugs, including those acting on the dopaminergic and serotonergic system (1, 2).

Utilizing the Neurocart in the pediatric age group would be particularly attractive, as it provides a non-invasive method of gathering data on the size and the time course of an effect in the setting of a drug trial. Minimal risk and burden are a requirement for drug trials in minors as stated by the International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use (3). With a legal framework now in place forcing industry to evaluate new drugs across all pediatric age groups (4), the need for validated and non-invasive biomarkers in the field of pediatric drug research is substantial.

In order to evaluate the suitability of the Neurocart test-battery in the pediatric age group, we performed a randomized placebo-controlled trial in children with Attention-Deficit Hyperactivity Disorder (ADHD) currently receiving treatment with methylphenidate (MPH). Methylphenidate was considered a good candidate for this study, as measurable effects on several tasks incorporated in the Neurocart were anticipated based on

previous experience with compounds with an effect on the dopaminergic system, and importantly, methylphenidate treatment can be temporarily withdrawn without any apparent risks to the patients involved. In fact, the British National Institute for Health and Clinical Excellence (NICE) guideline suggests to periodically suspend treatment in order to assess the child's condition while off medication (5). ADHD is a behavioral disorder characterized by symptoms of inattention, hyperactivity and impulsivity. It is one of the more common psychiatric disorders in childhood, with an estimated prevalence of 5% in school-age children (6). A leading theory postulates a deficit in behavioral inhibition, resulting in disruption of working memory, motor control, and sustained attention (7). Imaging studies in ADHD have shown hypoactivity in the prefrontal cortex and the anterior cingulate (8, 9). These brain structures are modulated mainly by the catecholaminergic neurotransmitters. MPH is thought to exert its effect mainly through an increase of dopamine levels in the synaptic clefts in the prefrontal cortex and basal ganglia by blocking dopamine reuptake (10, 11). On this theoretical basis, one would expect measurable effects of methylphenidate to be distinguished from placebo in neurocognitive testing. Indeed, a recent review identified improvement of performance on a wide range of neurocognitive tasks varying from 50-83.3% of published studies. The authors noted considerable variability of results between studies, caused among others by methodological limitations and problems associated with repeated neurocognitive testing (12).

The current study provided an opportunity to gather useful data on a number of additional parameters. Previous studies have demonstrated EEG changes after prolonged treatment of ADHD with stimulant medication in children (13, 14). This study provided the opportunity to study acute pharmaco-EEG after administration of methylphenidate.

The stimulant action of methylphenidate is known to elicit a small but statistically significant rise in blood pressure (15, 16). Cardiovascular effects of stimulant medication have come under increased interest since concerns have been raised that these effects may expose treated children to an increased risk of cardiovascular mortality (17). Therefore, we monitored cardiovascular parameters, providing data on acute effects of methylphenidate on heart rate and blood pressure.

In order to establish a relationship between methylphenidate levels and observed effects, pharmacokinetic data are required. Blood sampling in the paediatric age group in the setting of a non-therapeutic study is undesirable. Previously published literature shows that methylphenidate can be measured in saliva of children (18).

With this study, we intended to demonstrate whether tasks incorporated in the Neurocart test battery are suitable for measuring acute methylphenidate effects in children with ADHD. By simultaneously obtaining saliva methylphenidate concentrations, we might be able to build a model establishing a pharmacokinetic-pharmacodynamic (PK-PD) relationship in a future analysis.

Subjects and Methods

This was a randomized, placebo-controlled, 2-way cross-over study. Twenty children aged 8-12 years, with a confirmed DSM-IV diagnosis of ADHD, currently receiving treatment with methylphenidate were eligible for participation. Children treated with other drugs with known psychotropic effects were excluded. The Dutch Central Committee on Research involving Human subjects approved the study protocol. In compliance with the Declaration of Helsinki, written informed consent was obtained from the parents or legal guardians of the participating patients.

STUDY DRUGS

Capsules containing 5 mg of methylphenidate hydrochloride and matching placebos were prepared by the hospital pharmacy of the Leiden

University Medical Center. Participating children continued on their usual dose of methylphenidate; an equivalent regimen was calculated in case of children treated with sustained release formulations. Participants were randomized to either 7 days placebo followed by 7 days methylphenidate or vice versa. Randomization was performed using a 2 by 2 Williams square randomization procedure.

NEUROCOGNITIVE TASKS

Two single-trial computerized versions of the classic *colour-word Stroop tasks* were presented to the test subjects (19). In the first trial, 20 coloured items are presented at random. The subjects were asked to respond as quickly and accurately as possible by pressing the keys 1, 2 or 3 on the numerical pad with the index finger, middle finger and ring finger of the dominant hand, corresponding with the correct answer. In the second trial, which appeared directly after the first trial, 20 colour and word pairs were presented randomly to the subject, forming either congruent or incongruent matches. The subjects were again asked to respond as fast as possible by pressing the keys 1, 2 or 3 on the numerical pad, corresponding with the correct answer. In both trials, reaction times and the number of correct responses were recorded.

The *left/right distraction task* is a parametric version of the Stroop colour-word response conflict task. The words Left and Right were displayed either at the left or the right side of a computer screen. Response instructions are to respond quickly (by pressing a corresponding button) to the meaning of the word irrespective of its location. The output parameters are the response time and the number of correct responses.

The *adaptive tracking test* is a pursuit-tracking task. A circle of known dimensions moves randomly about a screen. The test subject must try to keep a dot inside the moving circle by operating a joystick. If this effort is successful, the speed of the moving circle increases. Conversely, the velocity is reduced if the test subject cannot maintain the dot inside the circle. In contrast to non-adaptive tracking methods, this leads to a constant and individually adapted challenge throughout the procedure. Performance is scored after a fixed period. The adaptive tracking test was performed as originally described by Borland and Nicholson (20, 21), using customised equipment and software (Hobbs, 2004, Hertfordshire, UK). The average performance and the standard deviation of scores over a 3.5-minute period is used for analysis. This 3.5-minute period includes a run-in time of 0.5 minute, in this run-in time the data is not recorded.

The measurement of *saccadic eye movements* was originally described by Baloh et al (22). In this study we used the nystagmo stimulator from Nihon Kohden (Nihon Kohden Corporation, Tokyo, Japan) for stimulus display, the program for signal collection and the AD-converter from Cambridge Electronic Design (CED Ltd., Cambridge, UK), the amplification by Grass (Grass-Telefactor, An Astro-Med, Inc. Product Group, Braintree, USA) and the sampling and analysis scripts were developed at the CHDR (Leiden, the Netherlands). Disposable electrodes were applied on the forehead and beside the lateral canthi of both eyes of the subject for registration of the electro-oculographic signals. Head movements were minimised with the aid of a head support placed opposite the target. The target consists of an array of light emitting diodes on a bar, fixed at 50 cm in front of the head support. Saccadic eye movements were recorded for stimulus amplitudes of approximately 15 degrees to either side. Fifteen saccades were recorded with interstimulus intervals varying randomly between 3 and 6 seconds. Average values of latency (reaction time), saccadic peak velocity of all *correct* saccades and inaccuracy of all saccades were used as parameters. Saccadic inaccuracy was calculated as the absolute value of the difference between the stimulus angle and the corresponding saccade, expressed as a percentage of the stimulus angle.

The same system as used for saccadic eye movements was also used for measurement of *smooth pursuit*. For smooth pursuit eye movements, the target moves sinusoidally at frequencies ranging from 0.3 to 1.1 Hz, by steps of 0.1 Hz. The amplitude of target displacement corresponds to 22.5

degrees eyeball rotation to both sides. Four cycles were recorded for each stimulus frequency. The time in which the eyes are in smooth pursuit of the target were calculated for each frequency and expressed as a percentage of stimulus duration. The average percentage of smooth pursuit for all stimulus frequencies was used as parameter.

Finger tapping has been adapted from the Halstead Reitan Test Battery (23), and evaluates motor activation and fluency. In this test speed of finger tapping is measured for the index finger of the dominant hand; a session contains five 10 second trials. Feedback on performance is given by a counter in the centre of the screen, while the amount of taps of each 10 second trial is shown on the screen in between the trials. The space bar is used as tapping device. The volunteer is instructed to tap as quickly as possible with the index finger and to rest the wrist on the table. The mean tapping rate and the standard deviations for the dominant hand are used for statistical analysis.

The *body sway* meter allows measurement of body movements in a single plane, providing a measure of postural stability. Body sway is measured with an apparatus similar to the Wright ataxiameter (24). With a string attached to the waist, all body movements in the sagittal (forward/backward) plane over a period of 2 minutes are integrated and expressed as mm sway on a digital display. Subjects were instructed to keep the eyes closed to eliminate the contribution of vision to postural control. Before starting a measurement, subjects were asked to stand still and comfortably, with their feet approximately 10 cm part and their hands in a relaxed position alongside the body.

EEG

EEG recordings were made using gold electrodes, fixed with EC2 paste (Astromed) at Fz, Cz, Pz and Oz, with the same common ground electrode as for the eye movement registration (international 10/20 system). The electrode resistances were kept below 5 kOhm. EEG signals were obtained from leads Fz-Cz and Pz-Oz and a separate channel to record eye movements (for artifacts). The signals were amplified by use of a Grass 15LT series Amplifier Systems with a time constant of 0.3 seconds and a low pass filter at 100 Hz. Data collection and analysis were performed using customized CED and Spike2 for Windows software (Cambridge Electronics Design, Cambridge, UK). Per session eight consecutive blocks of eight seconds were recorded. The signal was AD-converted using a CED 1401 Power (Cambridge Electronics Design, Cambridge, UK) and stored on hard disk for subsequent analysis. Data blocks containing artifacts were identified by visual inspection and these were excluded from analysis. For each lead, fast Fourier transform analysis was performed to obtain the sum of amplitudes in the delta- (0.5-3.5 Hz), theta (3.5-7.5 Hz), alpha- (7.5-11.5 Hz) and beta- (11.5-30 Hz) frequency ranges.

VITAL SIGNS

Blood pressure and heart rate were recorded using automated oscillometry.

QUESTIONNAIRES

At the end of each treatment week, parents and teachers completed a validated ADHD questionnaire. The questionnaire consists of 18 items, rating core symptoms of ADHD on a scale of 0 (behavior does not occur) to 4 (behavior occurs very often) (25).

PHARMACOKINETICS

saliva samples were obtained using the Polyester Salivette swab system (Sarstedt AG, Nümbrecht, Germany). This is a commercially available product designed specifically for collection of saliva specimens. It contains a roll of polyester which is held in the oral cavity for several minutes. After

collection, the swabs were centrifuged and the saliva was stored at -80 degrees Celsius until analysis. Analysis was performed on a validated GC/MS assay with a lower limit of quantitation (LLOQ) of 3 ng/mL. Samples measured below the LLOQ were set at 50% of the LLOQ (ie 1.5 ng/mL) for analysis purposes.

ASSESSMENT SCHEDULE

On day 7 of each of the study weeks, one or two assessments were performed before administration of the morning dose of methylphenidate, after which assessments were performed according to the schedule as represented in figure 1.

STATISTICAL ANALYSIS

a mixed model analysis of variance was performed to establish whether significant treatment effects could be detected on pharmacodynamic endpoints. The analysis was performed with fixed factors: treatment, time, occasion and treatment by time; random factors: subject, subject by treatment and subject by time. The averaged pre-dose measurements were used as covariate in the analysis. The pre-values were also analysed seperately, with the same model, to investigate a possible long-term effect of the MPH of the previous week. Least Square means estimates were calculated, as were contrasts between the two treatment conditions with 95% confidence intervals and p-values of the contrasts. Body sway results were log-transformed prior to analysis to correct for non-normal distribution of the data. A post-hoc analysis was performed on cardiovascular measurements, including only measurements 60-120 minutes post-dose, as the largest effect size was anticipated around maximum MPH concentrations. A second post-hoc analysis was performed to establish the effect of age on the results of different tasks. This analysis was performed with a mixed model regression analysis of age on a task (fixed factor: treatment; random factor: treatment by age, subject; covariate: age). The slope of the regression line of age on the respective variables was calculated, a slope significantly different from 0 would indicate an age effect. Finally, data from the current study were compared with data from 15 healthy children aged 8-12 years obtained in a previous study (Klein et al, submitted). For practical purposes the first three post-dose measurements from this study were compared with three consecutive measurements obtained in healthy children. The data were analyzed with a repeated measures model analysis of variance, with group, time and group by time as fixed factors, and time as repeated factor within subjects with a compound symmetry covariance structure and age as covariate. Contrasts between the three groups (ADHD on methylphenidate, ADHD on placebo, and healthy children) were calculated. Analyses were performed with SAS for windows version 9.1.2 (SAS Institute Inc., Cary, NC, USA). Results of pharmacokinetic analyses were calculated with Microsoft Excel 2007 (Microsoft co., Redmond, WA, USA) and are presented as mean ± SD.

Results

Twenty patients (18 male, 2 female; aged 10.5 ± 1.2 years) were recruited from several outpatient clinics. Of the 20 recruited patients, 10 were on a slow-release form of methylphenidate and 10 were on immediate release methylphenidate prior to study participation. The mean total daily dose prior to study enrolment was 26.6 ± 11.9 mg (range 10-54 mg). All patients completed the study.

Pharmacokinetic analyses showed methylphenidate concentration in saliva with a mean Cmax of 25.23 (±22.67) ng/mL at t=120 minutes post-dose, with an apparent elimination half-life of approximately 120 minutes (figure 2).

The analysis of the pre value showed no significant difference on any of the neurocognitive tasks. Results of the total analysis of neurocognitive

tasks are shown in table 1 and graphical representations of change from baseline are shown in figures 3a-e. Body sway was significantly reduced in the MPH condition. The eye movement tasks also showed a MPH effect: saccadic reaction time was reduced in the MPH condition and smooth pursuit performance increased. No differences were observed on saccadic inaccuracy and saccadic peak velocity. Adaptive tracking performance increased after MPH compared with placebo. Reaction times for colour naming in the Stroop task tended to increase during MPH; no difference was observed on reaction times in the conflict situation. The number of correct responses tended to increase on MPH in both the Stroop basic condition and the conflict condition. No differences were observed in the number of correct responses during the left/right distraction tasks, a trend toward shorter reaction times was observed during MPH. A slight but statistically significant increase in finger tapping performance was observed in the MPH condition. Variability of tapping frequency (standard deviation of mean tapping rate), was not influenced by MPH.

Analysis of EEG data revealed no significant differences between the ADHD and placebo study days (data not shown).

Comparison of the data with the data from a study in healthy children showed statistically significant contrasts in the body sway and adaptive tracking task, with task results of children with ADHD treated with MPH moving toward results of healthy children (table 3 and figures 4a-e).

Cardiovascular measurements showed increased blood pressure and heart rate after MPH. An analysis using only measurements obtained 60-120 minutes post-dose showed larger contrasts (table 2 and figures 5a-c).

The ADHD questionnaires completed by parents and teachers revealed a clear MPH effect as expected. Mean symptom score as rated by parents (n=19) was 25.4 during MPH and 42.2 during placebo ($p<0.0001$). Mean symptom score as rated by teachers (n=14) was 17.9 during MPH and 37.6 during placebo ($p<0.0001$).

The post hoc analysis investigating whether task results were influenced by age showed a significant age effect on the adaptive tracking task (slope of the regression line: 1.90 ±0.89; $p<0.05$, figure 6). An age effect was not observed in the other tasks (data not shown).

Discussion

This study investigated acute effects of methylphenidate in children with ADHD as compared with placebo. The effects covered a range of neurocognitive functions, as well as EEG and cardiovascular parameters. Several neurocognitive tasks showed clear effects of methylphenidate. Effects on blood pressure and heart rate could also be demonstrated. Rather than performing a single measurement in a treatment- or placebo condition, multiple measurements were performed in the hours following drug administration, thereby providing effect profiles over time.

Postural stability increased during MPH treatment. A previous study in children with ADHD only demonstrated improvement of postural stability during a dual task condition (the second task being a cognitive task), not during a single task condition. This observation seemed to support the theory that MPH effect on postural stability might be mediated through effects on dopamine networks in the frontal and prefrontal cortex, which are implicated in working memory and dual tasking (26, 27). In this sense, the MPH-induced postural improvement in our study would be related to beneficial effects on mental concentration. Alternatively, MPH effect might be mediated through direct dopaminergic influence on brain areas involved in motor and balance control. The movement effects of dopaminergic medication are well known, for instance of increasing dopamine levels in the striatum in patients with Parkinson's disease. Effects on postural stability in patients treated with ADHD may be mediated through a similar pathway (26). Findings from our current study seem to support the latter theory, as MPH effects were observed without increased working

memory workload. In this case, improved postural stability could be a manifestation of reduced motor restlessness.

MPH effects on oculomotor control demonstrated in this study (both smooth pursuit eye movements and visually guided saccades) included faster saccade initiation, and the ability to maintain smooth pursuit at higher stimulus velocities. Saccadic inaccuracy and saccadic peak velocity were not affected by MPH. Impairments of oculomotor control, as well as MPH effects on oculomotor tasks in children with ADHD have been demonstrated in several studies (28-32). Oculomotor control is a complex process, controlled by a number of brain areas including the posterior parietal and frontal cortex, basal ganglia, cerebellum, superior colliculus and brain stem reticular formation (31). The frontal eye fields form a component of this network located in the precentral gyrus in the frontal lobe, an area with a high density of dopaminergic receptors (33). The frontal eye fields are involved in both visual fixation and saccade initiation, thus altered performance on smooth pursuit and saccadic reaction time might be anticipated when administering dopaminergic compounds such as MPH.

We also demonstrated an increase in adaptive tracking performance after MPH administration was demonstrated. Adaptive tracking requires several skills including sustained attention, eye-hand motor coordination, and fine motor skills. Several studies using a pursuit task similar to our adaptive tracking task were performed in ADHD children and normal controls, demonstrating impaired performance in ADHD children (34, 35). Abilities such as fine motor coordination, nonverbal working memory and planning have been attributed to the broader concept of executive functioning, which is seemingly mediated by the prefrontal lobes of the frontal cortex (7, 36). Again, increased performance after MPH treatment as demonstrated in our study may be attributed to increased dopaminergic tone in the frontal cortex.

The left/right distraction task and the Stroop Color-Word task are two examples of neurocognitive tests targeting the phenomenon of response interference. When a stimulus with a single dimension is presented (e.g. a coloured rectangle), response time is faster than with a stimulus containing two conflicting or discongruent dimensions. (e.g. the word blue printed in red ink). Lower scores on the two-dimensional discongruent condition compared with the unidimensional stimulus reflect the interference effect, which is presumably caused by response conflict (37). When comparing children with ADHD to normal controls, a meta-analysis showed higher naming speed in the unidimensional condition in controls than in children with ADHD. Differences in the interference effect were inconsistent (37). Studies looking at MPH effects on Stroop performance in children with ADHD generally observed faster colour and word naming (single dimension stimulus), and inconsistent results on Stroop interference (38-41). No significant MPH effects were observed in the parameters studied in our study. Of note, the response times in the Stroop basic colour-naming condition tended to increase rather than decrease, contrary to previous findings. With essentially unchanged reaction times in the conflict condition, the smaller difference between the two conditions under MPH is caused by slower colour-naming rather than response interference. This could be related to reduced impulsivity. However, these observations need to be interpreted with caution, as they contradict previously published studies and differences were not statistically significant.

A small but statistically significant increase in tapping performance was observed during MPH. Motor timing in tapping tasks has been shown to be impaired in ADHD as compared to controls, and MPH has been shown to improve motor timing (42-44). The increased tapping performance observed in our study may be attributed to enhanced timing, or to better motor control. However, this is hard to judge, as we assessed maximum tapping frequency rather than paced or constant tapping.

This study failed to demonstrate any statistically significant EEG changes. EEG studies performed in children with ADHD have demonstrated differences as compared to controls, including increased theta in the frontal regions, and decreased alpha and beta activity in posterior regions.

Some studies have shown EEG normalization after MPH (13, 14). The small sample size number and the limited number of EEG-electrodes used may have reduced the ability to demonstrate EEG changes in this study.

Pharmacokinetic analyses demonstrated a profile consistent with previous work by other groups (18, 45). The variability is large, but this is to be expected with the heterogeneity of our study population (different doses used, and age range implicating different volumes of distribution between subjects). Before concentration-effect modelling can be undertaken, a pharmacokinetic model will be required including prediction of plasma levels. Ethical and practical issues withheld plasma sampling in our study. However, we expect to resolve this issue by studying plasma-saliva relations in a group of healthy volunteer adults, permitting future concentration-effect modelling.

Blood pressure (both diastolic and systolic) and heart rate were all shown to increase following MPH administration, with a maximum effect at 60-120 minutes post-dose. These findings were not unexpected, as methylphenidate is a stimulant drug with known adrenergic properties. Indeed, previous studies have repeatedly demonstrated a cardiovascular response to methylphenidate administration (15, 46). The design of our study with repeated measures following MPH administration demonstrates a clear concentration-effect relationship, with maximum blood pressure response occurring at the anticipated Tmax of MPH.

This study has shown, as expected, measurable effects of methylphenidate in children across a range of neurocognitive tasks. This study also demonstrates the feasibility of performing data-intensive studies in this age group with little burden or risk to participants. Based on these results, one could consider performing follow-up studies comparing for example the effect profiles of immediate-release methylphenidate and controlled-release methylphenidate. Assuming a valid PK model can be constructed with the obtained saliva PK samples, these studies could again include saliva PK monitoring, thereby enabling PK-PD modelling on the obtained dataset.

TABLE 1 Least square mean estimates of neurocognitive tasks during MPH or placebo, contrasts with 95% confidence interval, and change from baseline for placebo and MPH.

	LS Means Estimates		Contrasts	Change from baseline	
Variable	**Placebo**	**MPH**	**Placebo vs MPH (95% CI)**	**Placebo**	**MPH**
Body sway (mm)	751	475	-36.8% (-49.3%, -21.1%) p=0.0004	13.87%	-27.99%
Saccadic Inaccuracy (%)	6.7	6.5	-0.2 (-0.9, 0.5) p=0.5763	-0.3	-0.5
Saccadic Peak Velocity (deg/sec)	531.8	526.1	-5.8 (-18.2, 6.7) p=0.3364	-18.0	-23.8
Saccadic Reaction Time (sec)	0.237	0.223	-.014 (-.026, -.001) p=0.0305	0.011	-0.002
Smooth pursuit (%)	37.8	43.9	6.1 (2.3, 9.9) p=0.0034	-0.9	5.2
Adaptive tracking (%)	7.47	9.71	2.24 (0.85, 3.63) p=0.0032	-0.89	1.35
SD adaptive tracking (%)	2.57	2.65	0.08 (-0.21, 0.36) p=0.5560	0.09	0.17
L/R distraction correct	27.1	27.2	0.1 (-1.4, 1.6) p=0.9149	0.1	0.2
L/R distraction incorrect	3.3	3.5	0.2 (-1.1, 1.4) p=0.7870	-0.2	-0.0
L/R distraction rt correct (msec)	943.6	898.5	-45.0 (-119, 29.2) p=0.2176	7.5	-37.5
L/R distraction rt incorrect (msec)	758.9	730.9	-28.1 (-125, 68.8) p=0.5436	-20.7	-48.7
Stroop Basic correct	17.4	18.0	0.6 (-0.4, 1.6) p=0.2046	-0.8	-0.1
Stroop Basic correct rt (msec)	674.1	724.5	50.4 (-8.1, 108.9) p=0.0868	-123.4	-73.0
Stroop Conflict correct	16.9	17.6	0.7 (-0.4, 1.9) p=0.2048	-1.2	-0.5
Stroop Conflict correct rt (msec)	909.9	904.1	-5.8 (-95.7, 84.0) p=0.8927	-88.4	-94.3
Tap dominant hand	53.02	54.75	1.73 (0.55, 2.91) p=0.0081	-0.04	1.70
SD tap dominant hand	4.35	4.31	-0.04 (-0.99, 0.92) p=0.9339	0.46	0.42

TABLE 2 Results (least square means estimates) of cardiovascular measurements MPH or placebo, contrasts with 95% confidence interval, and change from baseline for placebo and MPH.

	LS Means Estimates		Contrasts	Change from baseline	
Variable	Placebo	MPH	Placebo vs MPH	Placebo	MPH
Heart rate-overall (bpm)	79.4	80.7	1.3 (-1.6, 4.3) p=0.3505	3.5	4.8
Heart rate-Tmax (bpm)	76.2	80.3	4.0 (0.5, 7.5) p=0.0255	0.3	4.4
Systolic blood pressure-overall (mmHg)	108.8	110.7	1.9 (-0.8, 4.7) p=0.1556	3.1	5.1
Systolic blood pressure-Tmax (mmHg)	106.5	111.1	4.5 (1.3, 7.8) p=0.0077	0.8	5.4
Diastolic blood pressure-overall (mmHg)	62.6	63.8	1.2 (-0.4, 2.8) p=0.1195	2.1	3.3
Diastolic blood pressure-Tmax (mmHg)	63.0	66.1	3.1 (1.0, 5.2) p=0.0043	2.5	5.6

TABLE 3 Calculated contrasts between children with ADHD on 2 treatment conditions and healthy children. Estimates of difference with 95% confidence intervals and the respective p-values are presented. (NS: not significant).

	Contrast	Estimate of difference (95% CI)	p-value
Body Sway	ADHD placebo – healthy	100.9 (49.8 to 169.5)%	< 0.0001
	ADHD MPH – healthy	36.4 (1.6 to 83.0)%	0.0394
	ADHD MPH – ADHD placebo	-32.1 (-41.1 to -21.8)%	< 0.0001
Saccadic reaction time	ADHD placebo – healthy	0.01 (-0.01 to 0.03) sec	NS
	ADHD MPH – healthy	-0.004 (-0.024 to 0.015) sec	NS
	ADHD MPH – ADHD placebo	-0.014 (-0.023 to -0.005) sec	0.0022
Smooth Pursuit	ADHD placebo – healthy	4.1 (-4.8 to 13.0)%	NS
	ADHD MPH – healthy	8.9 (0.1 to 17.8)%	0.0488
	ADHD MPH – ADHD placebo	4.8 (2.5 to 7.1)%	<0.0001
Adaptive Tracking	ADHD placebo – healthy	-8.07 (-10.85 to -5.29)%	<0.0001
	ADHD MPH – healthy	-5.98 (-8.76 to -3.20)%	<0.0001
	ADHD MPH – ADHD placebo	2.09 (0.90 to 3.28)%	0.0007
Tap dominant hand	ADHD placebo – healthy	-1.5 (-5.34 to 2.35)/10sec	NS
	ADHD MPH – healthy	0.02 (-3.82 to 3.86)/10 sec	NS
	ADHD MPH – ADHD placebo	1.52 (0.71 to 2.32)/10 sec	0.0003

FIGURE 1 Study assessment schedule. (time in minutes; the timepoint 0' indicates methylphenidate administration).

	-60'	-30'	0'	30'	60'	90'	120'	150'	180'	210'	240'	300'	360'
Saliva sample		⁝⁝		⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝
CNS block*	⁝⁝ **	⁝⁝			⁝⁝		⁝⁝		⁝⁝		⁝⁝		⁝⁝
cardiovascular		⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝	⁝⁝
EEG	⁝⁝	⁝⁝			⁝⁝		⁝⁝		⁝⁝		⁝⁝		⁝⁝

* The CNS block consists of consecutive administration of the neurocognitive tasks described in the methods section
** This block is intended as a training session. Some tasks (Saccadic Eye Movements and Stroop) were performed twice during this block to eliminate learning effects.

FIGURE 2 Methylphenidate levels in saliva (ng/ml) over time.

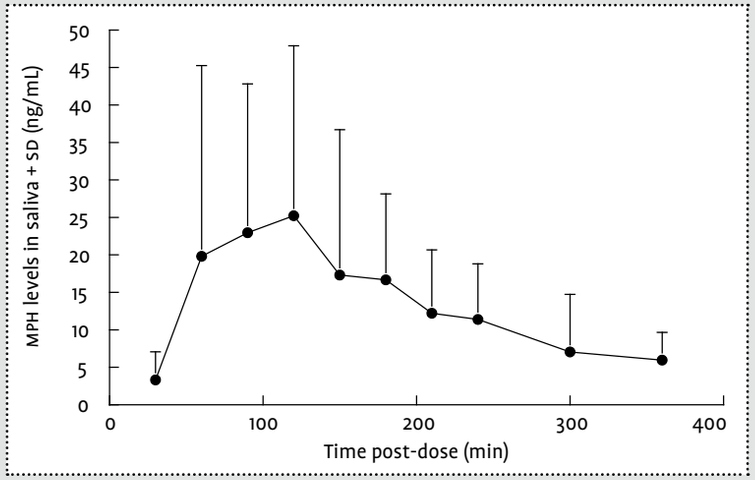
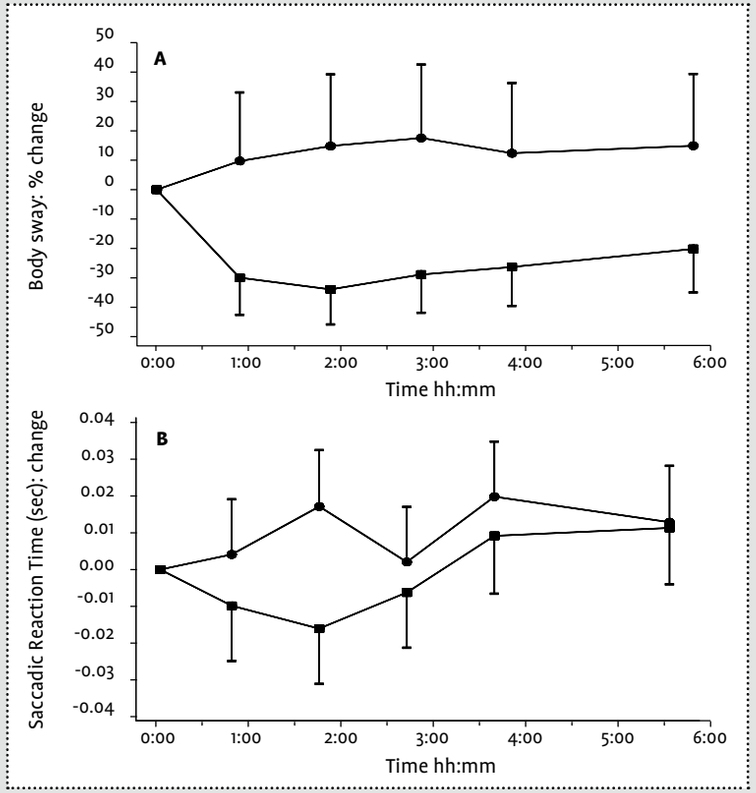


FIGURE 3A-E Change from baseline profile of neurocognitive tasks. Shown data are least square mean estimates, corrected for pre-dose measurements. Square: methylphenidate. Circle: placebo.



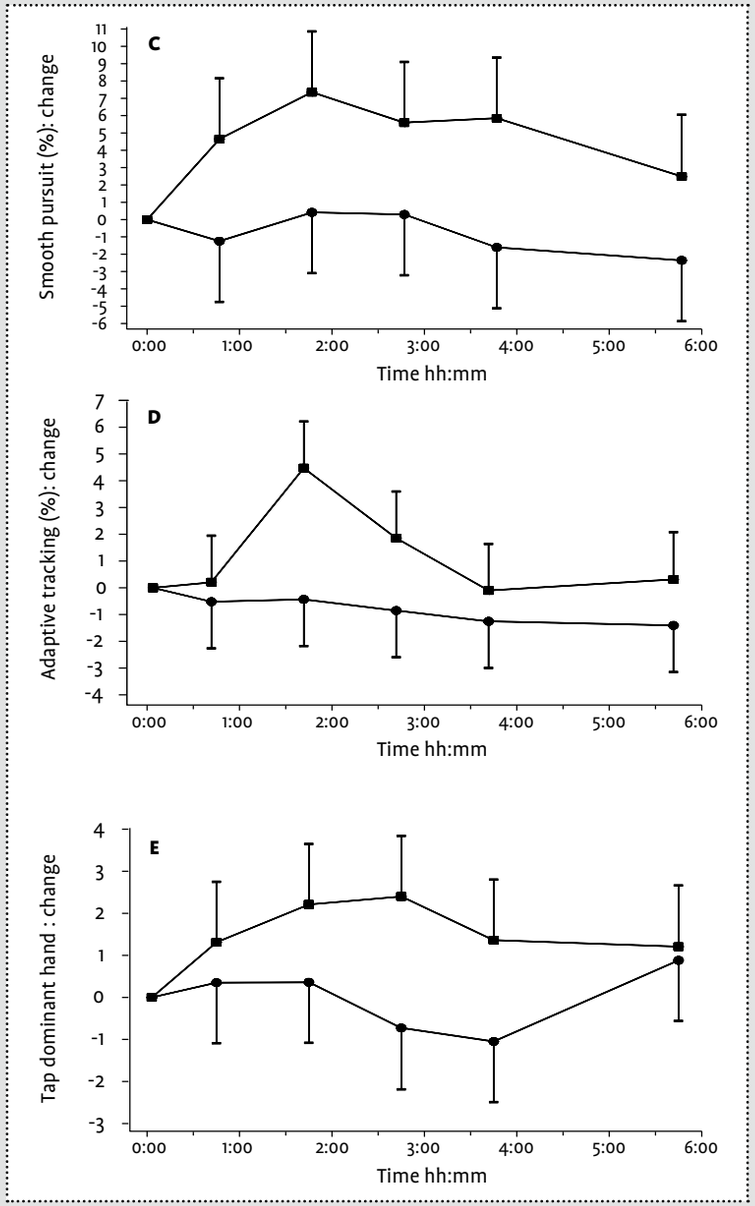
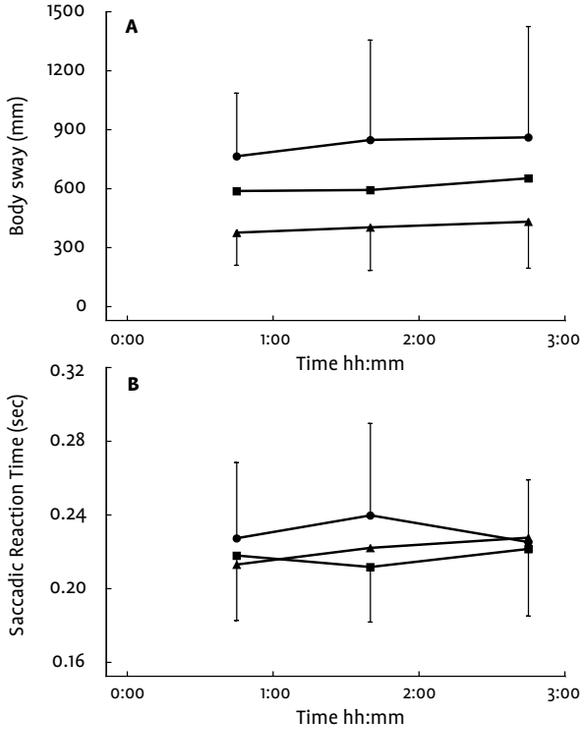


FIGURE 4A-E Comparison of the first 3 test runs after placebo (circle) and methylphenidate (square) to 3 consecutive test runs in healthy children (triangle) with SD error bars.



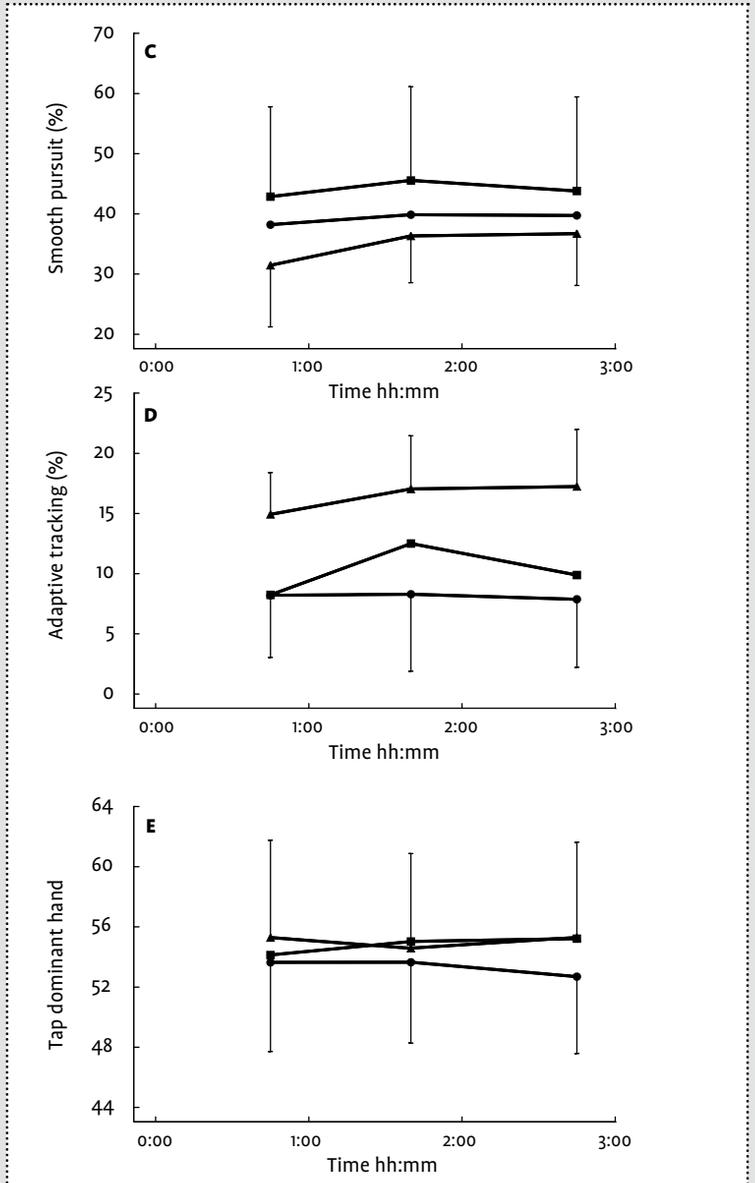


FIGURE 5A-C Change from baseline profile of vital signs. Shown data are least square means estimates, corrected for pre-dose measurements with SD error bars. Square: methylphenidate. Circle: placebo.

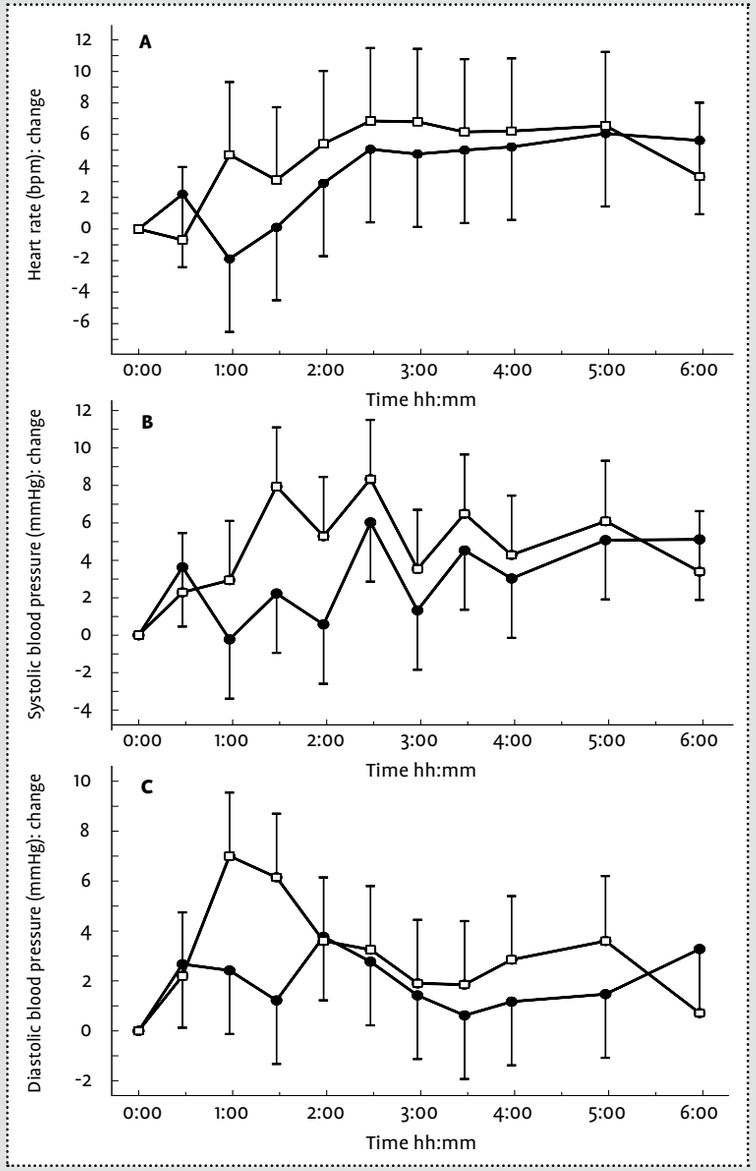
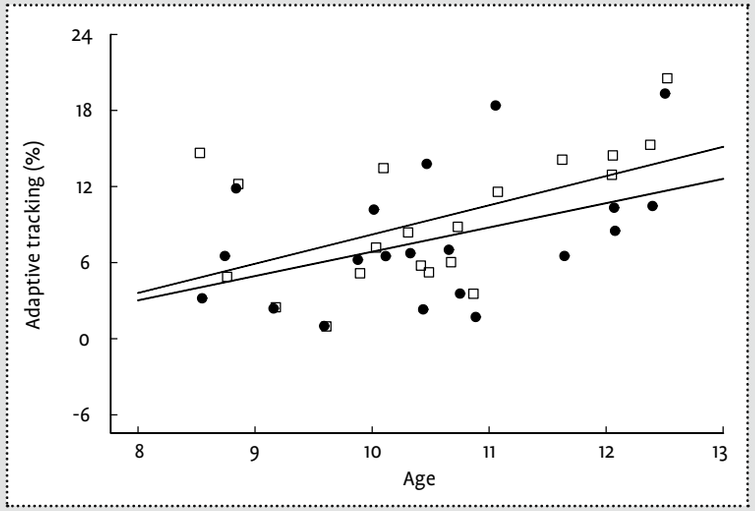


FIGURE 6 Regression line of age on adaptive tracking performance for both placebo (dots) and methylphenidate (squares).



1 de Visser SJ, van der Post J, Pieters MS, Cohen AF, van Gerven jm. Biomarkers for the effects of antipsychotic drugs in healthy volunteers. Br J Clin Pharmacol. 2001;51(2):119-32.

2 Dumont GJ, de Visser SJ, Cohen AF, van Gerven JM. Biomarkers for the effects of selective serotonin reuptake inhibitors (SSRIs) in healthy subjects. Br J Clin Pharmacol. 2005;59(5):495-510. DOI 10.1111/j.1365-2125.2005.02342.x

3 International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use. ICH Harmonised tripartite guideline E11-Clinical investigation of medicinal products in the pediatric population (2000).

4 Choonara I. Regulation of drugs for children in Europe. BMJ. 2007;335(7632):1221-2. DOI 10.1136/bmj.39400.376424.BE

5 TA 98 Methylphenidate, atomoxetine and dexamfetamine for attention deficit hyperactivity disorder (ADHD) in children and adolescents. London, UK: National Institute for Health and Clinical Excellence, 2006.

6 Polanczyk G, Rohde LA. Epidemiology of attention-deficit/hyperactivity disorder across the lifespan. Curr Opin Psychiatry. 2007;20(4):386-92. DOI 10.1097/YCO.0b013e3281568d7a

7 Barkley RA. Behavioral inhibition, sustained attention, and executive functions: constructing a unifying theory of ADHD. Psychol Bull. 1997;121(1):65-94.

8 Bush G, Frazier JA, Rauch SL, Seidman LJ, Whalen PJ, Jenike MA, et al. Anterior cingulate cortex dysfunction in attention-deficit/hyperactivity disorder revealed by fMRI and the Counting Stroop. Biol Psychiatry. 1999;45(12):1542-52.

9 Rubia K, Overmeyer S, Taylor E, Brammer M, Williams SC, Simmons A, et al. Hypofrontality in attention deficit hyperactivity disorder during higher-order motor control: a study with functional MRI. Am J Psychiatry. 1999;156(6):891-6.

10 Mehta MA, Owen AM, Sahakian BJ, Mavaddat N, Pickard JD, Robbins TW. Methylphenidate enhances working memory by modulating discrete frontal and parietal lobe regions in the human brain. J Neurosci. 2000;20(6):RC65.

11 Volkow ND, Wang GJ, Fowler JS, Ding YS. Imaging the effects of methylphenidate on brain dopamine: new model on its therapeutic actions for attention-deficit/hyperactivity disorder. Biol Psychiatry. 2005;57(11):1410-5. DOI 10.1016/j.biopsych.2004.11.006

12 Pietrzak RH, Mollica CM, Maruff P, Snyder PJ. Cognitive effects of immediate-release methylphenidate in children with attention-deficit/hyperactivity disorder. Neurosci Biobehav Rev. 2006;30(8):1225-45. DOI 10.1016/j.neubiorev.2006.10.002

13 Clarke AR, Barry RJ, Bond D, McCarthy R, Selikowitz M. Effects of stimulant medications on the EEG of children with attention-deficit/hyperactivity disorder. Psychopharmacology (Berl). 2002;164(3):277-84. DOI 10.1007/s00213-002-1205-0

14 Clarke AR, Barry RJ, McCarthy R, Selikowitz M, Johnstone SJ. Effects of stimulant medications on the EEG of girls with Attention-Deficit/Hyperactivity Disorder. Clin Neurophysiol. 2007;118(12):2700-8. DOI 10.1016/j.clinph.2007.08.020

15 Samuels JA, Franco K, Wan F, Sorof JM. Effect of stimulants on 24-h ambulatory blood pressure in children with ADHD: a double-blind, randomized, cross-over trial. Pediatr Nephrol. 2006;21(1):92-5. DOI 10.1007/s00467-005-2051-1

16 Stowe CD, Gardner SF, Gist CC, Schulz EG, Wells TG. 24-hour ambulatory blood pressure monitoring in male children receiving stimulant therapy. Ann Pharmacother. 2002;36(7-8):1142-9.

17 Nissen SE. ADHD drugs and cardiovascular risk. N Engl J Med. 2006;354(14):1445-8. DOI 10.1056/NEJMp068049

18 Greenhill LL, Cooper T, Solomon M, Fried J, Cornblatt B. Methylphenidate salivary levels in children. Psychopharmacol Bull. 1987;23(1):115-9.

19 Stroop JR. Studies of interference in serial verbal reactions. Journal of Experimental Psychology. 1935;18:643-62.

20 Borland RG, Nicholson AN. Comparison of the residual effects of two benzodiazepines (nitrazepam and flurazepam hydrochloride) and pentobarbitone sodium on human performance. Br J Clin Pharmacol. 1975;2(1):9-17.

21 Borland RG, Nicholson AN. Visual motor co-ordination and dynamic visual acuity. Br J Clin Pharmacol. 1984;18 Suppl 1:69S-72S.

22 Baloh RW, Sills AW, Kumley WE, Honrubia V. Quantitative measurement of saccade amplitude, duration, and velocity. Neurology. 1975;25(11):1065-70.

23 Andrew JM. Delinquents and the Tapping Test. J Clin Psychol. 1977;33(3):786-91.

24 Wright BM. A simple mechanical ataxia-meter. J Physiol. 1971;218 Suppl:27P-8P.

25 Scholte EM, van der Ploeg JD. Handleiding ADHD Vragenlijst (Dutch). Houten, the Netherlands: Bohn Stafleu van Loghum; 2005.

26 Jacobi-Polishook T, Shorer Z, Melzer I. The effect of methylphenidate on postural stability under single and dual task conditions in children with attention deficit hyperactivity disorder – a double blind randomized control trial. J Neurol Sci. 2009;280(1-2):15-21. DOI 10.1016/j.jns.2009.01.007

27 Leitner Y, Barak R, Giladi N, Peretz C, Eshel R, Gruendlinger L, et al. Gait in attention deficit hyperactivity disorder : effects of methylphenidate and dual tasking. J Neurol. 2007;254(10):1330-8. DOI 10.1007/s00415-006-0522-3

28 Bala SP, Cohen B, Morris AG, Atkin A, Gittelman R, Kates W. Saccades of hyperactive and normal boys during ocular pursuit. Dev Med Child Neurol. 1981;23(3):323-36.

29 Bylsma FW, Pivik RT. The effects of background illumination and stimulant medication on smooth pursuit eye movements of hyperactive children. J Abnorm Child Psychol. 1989;17(1):73-90.

30 Klein C, Jr Fischer B, Fischer B, Hartnegg K. Effects of methylphenidate on saccadic responses in patients with ADHD. Exp Brain Res. 2002;145(1):121-5. DOI 10.1007/s00221-002-1105-x

31 Munoz DP, Armstrong IT, Hampton KA, Moore KD. Altered control of visual fixation and saccadic eye movements in attention-deficit hyperactivity disorder. J Neurophysiol. 2003;90(1):503-14. DOI 10.1152/jn.00192.2003

32 O'Driscoll GA, Depatie L, Holahan AL, Savion-Lemieux T, Barr RG, Jolicoeur C, et al. Executive functions and methylphenidate response in subtypes of attention-deficit/hyperactivity disorder. Biol Psychiatry. 2005;57(11):1452-60. DOI 10.1016/j.biopsych.2005.02.029

33 Brown RM, Crane AM, Goldman PS. Regional distribution of monoamines in the cerebral cortex and subcortical structures of the rhesus monkey: concentrations and in vivo synthesis rates. Brain Res. 1979;168(1):133-50.

34 Rommelse NN, Altink ME, Oosterlaan J, Buschgens CJ, Buitelaar J, De Sonneville LM, et al. Motor control in children with ADHD and non-affected siblings: deficits most pronounced using the left hand. J Child Psychol Psychiatry. 2007;48(11):1071-9. DOI 10.1111/j.1469-7610.2007.01781.x

35 Slaats-Willemse D, de Sonneville L, Swaab-Barneveld H, Buitelaar J. Motor flexibility problems as a marker for genetic susceptibility to attention-deficit/hyperactivity disorder. Biol Psychiatry. 2005;58(3):233-8. DOI 10.1016/j.biopsych.2005.03.046

36 Barkley RA. Issues in the diagnosis of attention-deficit/hyperactivity disorder in children. Brain Dev. 2003;25(2):77-83.

37 van Mourik R, Oosterlaan J, Sergeant JA. The Stroop revisited: a meta-analysis of interference control in AD/HD. J Child Psychol Psychiatry. 2005;46(2):150-65. DOI 10.1111/j.1469-7610.2004.00345.x

38 Bedard AC, Ickowicz A, Tannock R. Methylphenidate improves Stroop naming speed, but not response interference, in children with attention deficit hyperactivity disorder. J Child Adolesc Psychopharmacol. 2002;12(4):301-9. DOI 10.1089/104454602762599844

39 Langleben DD, Monterosso J, Elman I, Ash B, Krikorian G, Austin G. Effect of methylphenidate on Stroop Color-Word task performance in children with attention deficit hyperactivity disorder. Psychiatry Res. 2006;141(3):315-20. DOI 10.1016/j.psychres.2005.09.007

40 Leitner Y, Doniger GM, Barak R, Simon ES, Hausdorff JM. A novel multidomain computerized cognitive assessment for attention-deficit hyperactivity disorder: evidence for widespread and circumscribed cognitive deficits. J Child Neurol. 2007;22(3):264-76. DOI 10.1177/0883073807299859

41 Scheres A, Oosterlaan J, Swanson J, Morein-Zamir S, Meiran N, Schut H, et al. The effect of methylphenidate on three forms of response inhibition in boys with AD/HD. J Abnorm Child Psychol. 2003;31(1):105-20.

42 Ben-Pazi H, Gross-Tsur V, Bergman H, Shalev RS. Abnormal rhythmic motor response in children with attention-deficit-hyperactivity disorder. Dev Med Child Neurol. 2003;45(11):743-5.

43 Rubia K, Noorloos J, Smith A, Gunning B, Sergeant J. Motor timing deficits in community and clinical boys with hyperactive behavior: the effect of methylphenidate on motor timing. J Abnorm Child Psychol. 2003;31(3):301-13.

44 Tiffin-Richards MC, Hasselhorn M, Richards ML, Banaschewski T, Rothenberger A. Time reproduction in finger tapping tasks by children with attention-deficit hyperactivity disorder and/or dyslexia. Dyslexia. 2004;10(4):299-315. DOI 10.1002/dys.281

45 Marchei E, Farre M, Pellegrini M, Garcia-Algar O, Vall O, Pacifici R, et al. Pharmacokinetics of methylphenidate in oral fluid and sweat of a pediatric subject. Forensic Sci Int. 2010;196(1-3):59-63. DOI 10.1016/j.forsciint.2009.12.038

46 Ballard JE, Boileau RA, Sleator EK, Massey BH, Sprague RL. Cardiovascular responses of hyperactive children to methylphenidate. JAMA. 1976;236(25):2870-4.

CHAPTER 7

Evaluation of a bedside device to assess the activated partial thromboplastin time for heparin monitoring in infants

Blood Coag Fibrinolysis, In Press

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Abstract

OBJECTIVE To determine the relationship between the activated partial thromboplastin time (aPTT) measured with a standard laboratory assay and the aPTT measured with a bedside device in infants on heparin therapy after cardiothoracic surgery.

METHODS Twenty infants aged <1 year who were on heparin therapy were included. Exclusion criteria were prematurity, dysmaturity and the use of anticoagulants other than heparin. Nineteen samples were obtained from 4 adults in intensive care who were on heparin. The aPTT values were analyzed with the Coaguchek Pro/DM® bedside device (aPTTbed) and compared with the aPTT values obtained from the laboratory Electra 1800C coagulation analyzer (aPTTlab). Correlation analysis was performed by linear regression. The agreement was calculated using Bland-Altman analysis.

RESULTS The correlation coefficient of samples obtained from infants was lower (r=0.48) compared with samples from adults (r=0.85). A substantial positive bias (27 sec) and scatter (95% CI: -11; +65 sec) was found. The bias showed a genuine trend to increase at higher aPTT values (r = 0.90; p<0.001).

CONCLUSIONS The bedside device overestimates the aPTT in infants treated with heparin. The disagreement between the bedside device and laboratory increases at higher aPTTs. Bedside devices should not be used to monitor heparin therapy in infants in intensive care.

Introduction

Effective antithrombotic therapy is important in treatment and prevention of thromboembolic disease. This is especially important in the pediatric setting, since it has been shown that the incidence of thromboembolic disease as a secondary complication of major primary illness, such as congenital heart disease, is increasing (1). Unfractionated heparin (in this report named heparin) is widely used as anticoagulant in pediatric patients (2).

Although it is acknowledged that laboratory testing of activated partial thromboplastin time (aPTT) has some disadvantages, it is still considered the gold standard for monitoring heparin therapy. However, laboratory testing has some disadvantages, such as being time-consuming and requiring a relatively large amount of blood. These features especially apply for infants in conditions that require frequent monitoring as in the setting of a pediatric intensive care unit (PICU).

Point-of-care methodology ('bedside-testing') may be a useful alternative in the pediatric setting, not only for aPTT measurement, but for measurement of any biological marker in pediatric patients as it requires only 1 drop of blood, returns results rapidly and is less invasive than conventional methodology. However, before this methodology can be used in the clinical setting it has to be validated rigorously. Validation of bedside coagulation monitoring devices is of particular importance in young infants, as the hemostatic system in childhood differs from adults (3).

The primary objective in this study is to determine the relationship between the aPTT measured with the routine laboratory assay and the aPTT measured with a bedside device (Coaguchek Pro/DM®, Roche Diagnostics) in infants on heparin therapy after cardiothoracic surgery. In addition, the relationship between these parameters was determined in adult patients on heparin therapy to investigate a possible influence of the immaturity of the coagulation system in infants.

Materials and methods

STUDY

This was an open observational study. The Medical Ethics Committee of Leiden University Medical Center (LUMC) approved the study protocol. The procedures followed were in accordance with the Helsinki Declaration. Written informed consent was obtained from the parents or legal guardians of the pediatric patients or from the adult patients.

SUBJECTS

Infants, below 1 year of age, who were admitted to the PICU of the LUMC after cardiothoracic surgery and who were on heparin therapy or in whom heparin therapy was discontinued (according to the routine clinical care indicated for the clinical condition) were included. Exclusion criteria were prematurity, dysmaturity and the use of anticoagulants other than heparin. As this study was carried out during the routine clinical care of the patients, all other medication necessary for the condition of the patient was allowed. In addition, 20 samples were collected from 4 adult patients admitted to the Centre for Intensive Care of LUMC who were on heparin therapy.

SAMPLING AND ANALYSIS

For infants who were on heparin therapy a maximum of 9 samples was allowed. At maximum, 4 bedside samples were drawn from infants in whom heparin therapy was discontinued. These samples were taken according to the algorithm used in our hospital for the routine clinical evaluation of the aPTT. The samples were taken over a period of 36-48 hours. A maximum of 5 samples was taken from adult patients. Blood samples for the routine laboratory aPTT measurement (aPTTlab) determination were taken according to the current practice of LUMC using a standard tube with citrate as anticoagulant. A routine blood sample for the laboratory and one drop of blood for the bedside aPTT measurement (aPTTbed) were drawn subsequently from the same (non-heparin coated) catheter. Thus identical samples were obtained. The laboratory measurements were performed on the Electra 1800C automated coagulation analyzer (Instrumentation Laboratory BV, Breda, The Netherlands). To measure aPTTbed the Coaguchek Pro/DM® coagulation device (Roche Diagnostics, Basel, Switzerland) was used according to manufacturer's instructions. The aPTT normal range is 21-41 seconds (sec) and has a measurement range from 18-150 sec. The within-day and between-day precision (CV) for assessment of aPTT is reported to range from 3-7% and from 4-8%, respectively (4, 5). The precision of the Coaguchek for duplicate samples was evaluated by determining the percent mean absolute error and varies between 5.8% for heparin-treated individuals and 17.8% for non-treated individuals according to the manufacturer.

Within-run and run-to-run precision of the Coaguchek Pro/DM® were monitored using quality controls at two levels (level 1 corresponding to healthy individuals, level 2 in the therapeutic range of heparin therapy).

DATA ANALYSIS

The correlation between the values for each pair of results (aPTTlab and aPTTbed) was investigated by linear regression. The agreement between aPTTlab and aPTTbed was calculated according to the method of comparison described by Bland and Altman (6). The differences were plotted against the average value of both methods. In the plot, three observations can be made: the average difference (bias) between the two methods, the variability of the differences, as shown by the scatter, and a possible trend in the difference. The latter observation indicates whether a tendency of the mean difference to increase or decrease with increasing magnitude exists (7). It should be noted that, if a trend is observed, the magnitude of the average difference is dependent on the data range. The slopes of the

regression lines were also calculated, because the data range influences correlation coefficients. A regression line with a non-zero correlation and slope will reflect a genuine change in the difference with increasing magnitude.

The data were viewed as replicated data in pairs. If paired measurements were repeated in the same patient, they were treated as independent pairs, as the underlying true aPTT value may have changed over time.

Results

A total of 62 samples were obtained from 20 infants. The majority of samples (90%) were collected from 19 infants in whom heparin therapy was discontinued at the PICU after cardiothoracic surgery. Five samples were drawn from an infant currently on heparin therapy. A total of 19 samples were obtained from 4 adults who were on heparin therapy.

The aPTT values measured in the laboratory were within a reasonable therapeutic range of 20-60 sec. No significant differences were observed between the aPTTlab values determined in child samples and adult samples. The mean and coefficient of variation for within-run precision were 55.7s (4.7%) and 116.1s (4.5%) for the two aPTT quality control levels tested. For run-to-run precision these values were 56.2s (5.8%) and 116.8s (3.8%).

The correlation between the values for each pair of results (aPTTlab and aPTTbed) is shown in figure 1. The aPTTlab values correlated reasonably well with the aPTTbed values for the entire data set (r = 0.59). The slope of the linear equation fitted to the data was in the same order of magnitude for the samples collected in infants (slope: 1.9) and adults (slope: 2.7). However, the correlation coefficient of samples obtained from infants was lower (r=0.48) compared with the correlation coefficient of adult samples (r=0.85).

Results of the bias analysis are shown in figure 2. A substantial positive bias (27 sec) and scatter (95%CI: -11; +65 sec) was found. There was no difference in bias for the samples obtained from infants (27 sec) and adults (25 sec). In addition, in both populations the bias showed a genuine trend to increase at higher aPTT values (r = 0.90; p<0.001).

Discussion

This study compared a routine laboratory assay with a bedside device (Coaguchek Pro/DM®, Roche Diagnostics) for measurement of aPTT values in infants on heparin therapy after cardiothoracic surgery. In addition, these methods for measuring aPTT were compared in adult patients on heparin. The majority of blood samples were obtained from infants after cessation of heparin therapy. However, the aPTT values measured in the laboratory were within the therapeutic range of 20-60 sec and reflect the commonly used treatment goal of 2 to 3 times prolongation of aPTT above baseline. This enabled us to determine the ability of point-of-care monitoring to control heparin therapy in infants. It also avoided the problems associated with assessment of heparin activity when given in high doses. There are indications that in these situations both the aPTT and the Activated Clotting Time (ACT) become unreliable (8).

Despite point-of-care aPTT monitoring becoming widely available, little data is available evaluating its accuracy in the pediatric population. The American College of Chest Physicians, at the Eighth Consensus Conference on Antithrombotic and Thrombolytic Therapy, concluded that accuracy of point-of-care monitors for heparin therapy had thus far not been established (9). A review concluded that clinical application of point-of-care aPTT testing is currently limited (10). The main finding of this study was that the bedside device substantially overestimated the aPTT in infants treated with heparin. This disagreement increased at higher aPTTs. A previously published study (with a different bedside device) in neonates reported underestimation at lower aPTT levels, and

overestimation at higher levels (11). Similar observations were made in a study evaluating a bedside device measuring activated clotting time (12).

In contrast to our current findings, we have shown in a previous study in adult patients on heparin therapy that the bedside monitor underestimated aPTT values, and this underestimation became more prominent at higher aPTT values (13). This difference in results could be due to the fact that in our previous study the adult patients received a single dose of heparin intravenously, whereas in the present study both adult and pediatric patients received heparin by continuous intravenous infusion (14). However, an alternative explanation can also be given. In adult patients, a number of studies have assessed the agreement of a point-of-care monitor and laboratory assay for the aPTT values during heparin therapy. These studies have reported excellent correlations (4, 5, 15-17) as well as large differences (18-20) between bedside and laboratory aPTT values. In general, ambiguous results have been reported for adults. Thus, it may also be possible that the different observations between the current results and our previous observations reflect this ambiguity.

A variety of factors is known to influence the aPTT measurement system and may be responsible for the difference between bedside and laboratory aPTT values during heparin therapy. The difference in heparin sensitivity between methods is a potential confounding factor in any comparative study of aPTT monitoring techniques. The type of measurement system, handling of blood samples and composition of the reagent, are examples of factors that affect aPTT analysis (21). Commercial aPTT reagents vary in responsiveness to heparin. Laboratory and bedside aPTT measurement systems are calibrated with different reagents (22). The sensitivity of the aPTT test to these external factors causes the test to be inherently inaccurate. Despite these limitations, the aPTT will remain an important assay in the monitoring of heparin therapy, as it is widely available and clinicians are familiar with its use (23).

Naturally, patient factors also influence aPTT measurements. This may particularly be the case in pediatric patients. It might be argued

that the discrepancy found in this study is due to the immaturity of the blood coagulation system in young infants, as heparin interaction with an immature coagulation system is different from the mature situation in adults. Heparin has a powerful anticoagulant mode of action (24, 25). Briefly, heparin produces anticoagulation primarily by increasing the affinity of binding of AT to thrombin rendering the thrombin unavailable for activation of coagulation. Heparin therapy is commonly and increasingly used in pediatric patients (26). Importantly, there is an unclear influence of age on heparin anticoagulant activities and pharmacokinetics. Increased sensitivity as well as resistance to heparin have been reported in children (27). Increased sensitivity may be explained by the fact that, during childhood, the capacity of plasma to generate thrombin is both delayed and decreased compared with adults (28). Plasma concentrations of AT are significantly decreased in many pediatric patients leading to a lower effect of heparin and reflecting the immaturity of the human coagulation system in infancy (29). In addition, pediatric patients require more heparin to achieve the adult therapeutic range, because of the higher clearance of heparin in infants and different proportion of intra- and extracellular space in children (30).

In this study, the only finding suggesting that the relationship between the laboratory and bedside measurement is different in infants and adults is the difference in correlation between the two parameters in the two populations. This difference in correlation is too small to conclude that there is a difference between laboratory and bedside measurement in infants and adults. Moreover, results from the Bland-Altman analysis showed no difference in bias for the samples obtained from infants and adults and the bias showed a similar trend to increase at higher aPTT values in both populations.

To date, guidelines for heparin dosage in the pediatric population are extrapolated and adapted from adult guidelines. In addition, nomograms for aPTT levels, which were established for adult patients, have now been adapted, tested and modified for infants (2, 31). Nomograms may

be helpful in using different measurement methods interchangeably. Although the laboratory aPTT values correlated reasonably well with the point-of-care aPTT values, this correlation was too low for establishment of a nomogram.

Conclusions

The bedside device evaluated in this study substantially overestimates the aPTT in infants treated with heparin. The disagreement between the bedside device and laboratory increased at higher aPTTs, and was of to great a magnitude to justify safe clinical use. At present, bedside aPTT measurement should not be used to monitor heparin therapy in infants at the intensive care unit. It remains to be determined if this overestimation of the aPTT leads to under medication of the patient. Laboratory aPTT measurement should remain the primary method to monitor heparin therapy in infants at the intensive care unit and, at present, certainly not be replaced by bedside devices. Despite the failure of this point-of-care device in producing accurate results, we believe this study should not be read as a 'generic' disencouragement of developing point-of-care methodology, as the potential benefits for the pediatric population remain. It does however emphasize the need for thorough validation before application in clinical practice.

FIGURE 1 Correlation between the aPTT values measured with routine laboratory methodology and a bedside device in infants (•) and adults (□) on heparin therapy. The line indicates the best linear fit between the data points (r=0.59).

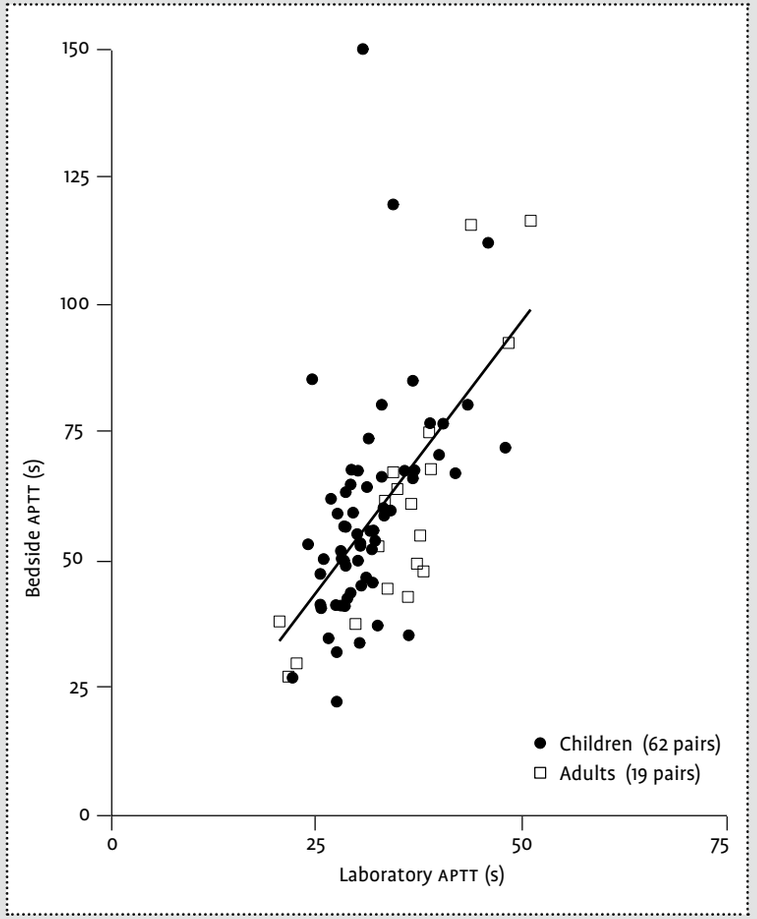
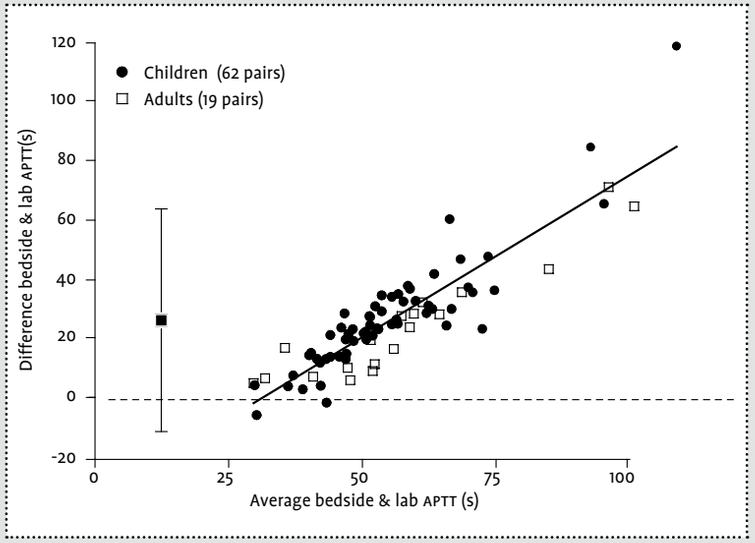


FIGURE 2 Bland-Altman plot for aPTT measurements. The scatter above the (dashed) zero line indicates overestimation of the aPTT by the bedside monitor. The solid line represents the regression line for the observed trend in the difference (p<0.001). The marker with error bars indicates mean difference and 95% limits of agreement.



1 Andrew M, Marzinotto V, Brooker LA, Adams M, Ginsberg J, Freedom R, et al. Oral anticoagulation therapy in pediatric patients: a prospective study. Thromb Haemost. 1994;71(3):265-9.

2 Andrew M, Marzinotto V, Massicotte P, Blanchette V, Ginsberg J, Brill-Edwards P, et al. Heparin therapy in pediatric patients: a prospective cohort study. Pediatr Res. 1994;35(1):78-83.

3 Andrew M, Paes B, Johnston M. Development of the hemostatic system in the neonate and young infant. Am J Pediatr Hematol Oncol. 1990;12(1):95-104.

4 Eiswirth G, Walch S, Bommer J. New bedside test for monitoring anticoagulation during hemodialysis. Artif Organs. 1998;22(4):346-8.

5 Ruzicka K, Kapiotis S, Quehenberger P, Handler S, Hornykewycz S, Michitsch A, et al. Evaluation of bedside prothrombin time and activated partial thromboplastin time measurement by coagulation analyzer CoaguCheck Plus in various clinical settings. Thromb Res. 1997;87(5):431-40.

6 Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;1(8476):307-10.

7 Bland JM, Altman DG. Comparing methods of measurement: why plotting difference against standard method is misleading. Lancet. 1995;346(8982):1085-7.

8 Owings JT, Pollock ME, Gosselin RC, Ireland K, Jahr JS, Larkin EC. Anticoagulation of children undergoing cardiopulmonary bypass is overestimated by current monitoring techniques. Arch Surg. 2000;135(9):1042-7.

9 Monagle P, Chalmers E, Chan A, DeVeber G, Kirkham F, Massicotte P, et al. Antithrombotic therapy in neonates and children: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). Chest. 2008;133(6 Suppl):887S-968S. DOI 10.1378/chest.08-0762

10 Spinler SA, Wittkowsky AK, Nutescu EA, Smythe MA. Anticoagulation monitoring part 2: Unfractionated heparin and low-molecular-weight heparin. Ann Pharmacother. 2005;39(7-8):1275-85. DOI 10.1345/aph.1E524

11 Tan K, Booth D, Newell SJ, Dear PR, Hughes C, Richards M. Point-of-care testing of neonatal coagulation. Clin Lab Haematol. 2006;28(2):117-21. DOI 10.1111/j.1365-2257.2006.00765.x

12 Racioppi L, Quinart A, Biais M, Nouette-Gaulain K, Revel P, Sztark F. Validation of a bedside activated clotting time test (Hemochron Jr II Signature) with low dose heparin therapy. Anaesthesia. 2009;64(4):430-4. DOI 10.1111/j.1365-2044.2008.05822.x

13 Kemme MJ, Faaij RA, Schoemaker RC, Kluft C, Meijer P, Cohen AF, et al. Disagreement between bedside and laboratory activated partial thromboplastin time and international normalized ratio for various novel anticoagulants. Blood Coagul Fibrinolysis. 2001;12(7):583-91.

14 Despotis GJ, Levine V, Joiner-Maier D, Joist JH. A comparison between continuous infusion versus standard bolus administration of heparin based on monitoring in cardiac surgery. Blood Coagul Fibrinolysis. 1997;8(7):419-30.

15 Chavez JJ, Weatherall JS, Strevels SM, Liu F, Snider CC, Carroll RC. Evaluation of a point-of-care coagulation analyzer on patients undergoing cardiopulmonary bypass surgery. J Clin Anesth. 2004;16(1):7-10. DOI 10.1016/j.jclinane.2003.03.004

16 Despotis GJ, Hogue CW, Jr., Santoro SA, Joist JH, Barnes PW, Lappas DG. Effect of heparin on whole blood activated partial thromboplastin time using a portable, whole blood coagulation monitor. Crit Care Med. 1995;23(10):1674-9.

17 Reiner JS, Coyne KS, Lundergan CF, Ross AM. Bedside monitoring of heparin therapy: comparison of activated clotting time to activated partial thromboplastin time. Cathet Cardiovasc Diagn. 1994;32(1):49-52.

18 Milos M, Coen D, Zadro R. Reliability of prothrombin and activated partial thromboplastin time determination on Coagucheck Pro DM. Point of Care. 2004;3:135-9.

19 Nuttall GA, Oliver WC, Jr., Beynen FM, Dull JJ, Murray MJ, Nichols WL. Intraoperative measurement of activated partial thromboplastin time and prothrombin time by a portable laser photometer in patients following cardiopulmonary bypass. Cardiothorac Vasc Anesth. 1993;7(4):402-9.

20 Reich DL, Yanakakis MJ, Vela-Cantos FP, DePerio M, Jacobs E. Comparison of bedside coagulation monitoring tests with standard laboratory tests in patients after cardiac surgery. Anesth Analg. 1993;77(4):673-9.

21 Gibaldi M, Wittkowsky AK. Contemporary use of and future roles for heparin in antithrombotic therapy. J Clin Pharmacol. 1995;35(11):1031-45.

22 Manzato F, Mengoni A, Grilenzoni A, Lippi G. Evaluation of the activated partial thromboplastin time (APTT) sensitivity to heparin using five commercial reagents: implications for therapeutic monitoring. Clin Chem Lab Med. 1998;36(12):975-80. DOI 10.1515/CCLM.1998.168

23 Eikelboom JW, Hirsh J. Monitoring unfractionated heparin with the aPTT: time for a fresh look. Thromb Haemost. 2006;96(5):547-52.

24 Hirsh J. Heparin. N Engl J Med. 1991;324(22):1565-74. DOI 10.1056/NEJM199105303242206

25 Hirsh J, Warkentin TE, Raschke R, Granger C, Ohman EM, Dalen JE. Heparin and low-molecular-weight heparin: mechanisms of action, pharmacokinetics, dosing considerations, monitoring, efficacy, and safety. Chest. 1998;114(5 Suppl):489S-510S.

26 Sutor AH, Massicotte P, Leaker M, Andrew M. Heparin therapy in pediatric patients. Semin Thromb Hemost. 1997;23(3):303-19. DOI 10.1055/s-2007-996103

27 Vieira A, Berry L, Ofosu F, Andrew M. Heparin sensitivity and resistance in the neonate: an explanation. Thromb Res. 1991;63(1):85-98.

28 Andrew M, Mitchell L, Vegh P, Ofosu F. Thrombin regulation in children differs from adults in the absence and presence of heparin. Thromb Haemost. 1994;72(6):836-42.

29 Andrew M, Vegh P, Johnston M, Bowker J, Ofosu F, Mitchell L. Maturation of the hemostatic system during childhood. Blood. 1992;80(8):1998-2005.

30 McDonald MM, Jacobson LJ, Hay WW, Jr., Hathaway WE. Heparin clearance in the newborn. Pediatr Res. 1981;15(7):1015-8.

31 Cruickshank MK, Levine MN, Hirsh J, Roberts R, Siguenza M. A standard heparin nomogram for the management of heparin therapy. Arch Intern Med. 1991;151(2):333-7.

CHAPTER 8

General Discussion

Overview

In this thesis, several aspects of pediatric pharmacology were covered. We have analyzed the effects of new American and European legislation, and concluded that although successful in triggering a rapid development of research infrastructure, labeling changes have occurred primarily in compounds used in the adult population. The clinical studies included in this thesis illustrate some of the issues playing a role in pediatric drug research. A study on the use of clonidine during an oral growth hormone stimulation test investigated pharmacokinetics (PK) and pharmacodynamics (PD) of clonidine in this setting, providing insight in the relation between clonidine plasma levels and (un)desired effects. We studied a neurocognitive test battery in both healthy children and children with attention deficit hyperactivity disorder (ADHD). In the ADHD study, saliva sampling was performed to enable PK-PD modeling in the future. These two studies provide examples of how existing methodology can be put to good use in the pediatric population, and how non-invasive measurement of drug levels can circumvent the need for intravenous sampling in a setting in which this would be undesirable and unpractical. Finally, a study on levothyroxine provides a typical example of the problems associated with suitable formulations being unavailable for specific age groups.

LEGISLATIVE EFFORTS

With powerful legislation having come into force in both Europe and the United States, aimed at increasing the amount of research performed in the field of pediatric pharmacology, a growing volume of studies involving children is observed. This is of course a desired effect, which is needed to attain the ultimate goal of increasing the amount of age-appropriate information about drugs prescribed to children. However, the approach taken by the legislators has been criticized, as the reward for pharmaceutical companies submitting research data (6-month patent extension) is quite substantial. We have demonstrated how 'big pharma' have exploited the new legislation, by obtaining pediatric exclusivity on blockbuster drugs used primarily by adults. In 2005, 5 out of the top 10 compounds by US sales saw their market protection extended by 6 months under pediatric exclusivity (1). These 5 entities (atorvastatin, simvastatin, omeprazole, lansoprazol and sertraline) were not necessarily associated with frequent use or a substantial need in the pediatric population. More recent data show a similar picture: the 8 top selling compounds in the US in 2011 (atorvastatin, clopidogrel, esomeprazole, aripiprazole, fluticasone/salmeterol, quetiapine, montelukast and rosuvastatin) all obtained pediatric exclusivity (IMS Health, 2012). The combined US sales figure of these 8 compounds amounts to 44.1 billion US dollars. The self-reported estimate of study costs to comply with a Written Request estimated by the Pharmaceutical Research and Manufacturers of America, are 5 to 35 million US dollars (2). It is thus unsurprising that cost to society of the 6-month patent extension is substantial. A recent study estimated the financial impact on the American Medicaid programme of patent extension of just 3 drug classes (statins, ACE inhibitors and SSRI's) at 430 million US dollars (3). Although these financial rewards could be seen as excessive, the regulation has been argued to be successful in expanding the volume of research performed and knowledge gaps being addressed (4). We do argue however that the cost to society and the reward to the industry are in disproportion.

The legislation was introduced in an era in which several 'blockbuster' drugs (e.g. statins, serotonin reuptake inhibitors, proton pump inhibitors) were covered by a patent, which enabled pharmaceutical companies to generate additional revenue during this extended market protection period. So perhaps it is not surprising that the effects of the legislation are mainly observed among the blockbuster drugs. And as 'few pediatric conditions have all the ingredients that would favor blockbuster therapies' (5), large commercial interests will not suddenly lead to big pharma investing in pediatric research for commercial reasons. One could argue that the

reward now offered to pharmaceutical companies could be downgraded over the coming years, while maintaining the obligation of including research in the pediatric population with newly submitted dossiers. At the same time, financial incentives for off-patent compounds could be increased further, as the largest need for safety and efficacy data lies with compounds from this group. Other suggested incentives include tax benefits, public funding and liability limitations (5). Both the American Food and Drug Administration (FDA) and European Medicines Agency (EMA) have long recognized the need for information on off-patent drugs, and have both published priority lists with off-patent compounds requiring additional research.

MAKING FORMULATIONS SUITABLE FOR CHILDREN

In addition to the existing problem of a lack of information concerning the active compounds, there is a lack of availability of formulations suitable for children, like liquids or chewable tablets (6). As there are many situations in which young children require oral administration of compounds (for example diuretics for a neonate with a congenital heart defect) unavailable as a liquid, hospital pharmacies often prepare extemporaneous oral liquids. This by definition creates a volume of unlicensed drug prescribing, which is undesirable as there are a lot of 'unknowns' surrounding the prepared formulation (like for example bioavailability and shelf life).

If a suitable formulation is available, it will often contain excipients like solvents, conservatives, flavourings, and pH correcting agents. In 2008, it was reported by the BBC that of 41 medicines available over-the-counter used in 3-year-olds in the UK, 40 contained additives banned from foods intended for 3-year-olds (5). For example, the oral acetaminophen (Sinaspril ®) formulation available in the Netherlands contains ethanol and propylene glycol, in amounts not specified in the product information leaflet. The Levothyroxin preparation described in Chapter 3 of this thesis was also determined to contain propylene glycol. There are no available pediatric data with regard to exposure limits of these excipients. Common practice is extrapolating recommended exposure from adults, which is probably inadequate, in analogy with similar extrapolation of drug doses. When these extrapolated data are used, excipient exposure can exceed the recommended limits, as has been demonstrated in a recent publication on excipient exposure in preterm infants (7), with levels of ethanol and propylene glycol exposure above the (extrapolated) exposure limits. Studies on the pharmacokinetics ('toxicokinetics') and safety of these excipients, like for example the recent work on propylene glycol (8, 9), is needed to establish the safety of formulations containing these excipients.

The problems associated with formulations unsuitable for children being used in clinical practice are illustrated in Chapter 3. In the Netherlands, levothyroxine for oral administration is only available as tablets. When administering levothyroxine to small children, tablets are crushed and dissolved in water. The obtained solution is then administered orally, or in case of preterms and neonates through a nasogastric feeding tube. The main findings of our study were firstly that the 'solution' is in fact a suspension, and secondly that drug particles stay behind in feeding tubes when administered through this route. Both obviously lead to uncertainty on the amount of active drug actually reaching the patient. By administering a levothyroxine solution holding a market registration for oral administration, these problems do not occur (10).

OBTAINING PHARMACOKINETIC DATA WITH MINIMAL BURDEN TO THE PATIENT

It can be challenging to find and/or create settings in which it is possible to gather adequate pharmacological data, without creating unacceptable levels of risk or burden to participating subjects. Such settings can be found however: over recent years, many studies have been performed

in neonatal and pediatric intensive care wards, where blood sampling is relatively easy as most patients have an arterial line. Large amounts of pharmacokinetic data have been generated by these studies, and illustrate how knowledge on frequently used compounds can be increased drastically, without violating the ethical limits in pediatric drug research. Examples include pharmacokinetic studies on propofol, acetaminophen and midazolam performed in surgical PICU's (11-13). A large multicenter study on the pharmacokinetics of anticonvulsants and antibiotics in asphyxiated infants also exploited the possibilities of pharmacokinetic sampling in an intensive care unit (14). However, many medicines are administered to children in situations were frequent sampling would constitute a burden and this forms an impediment for recruiting patients. New technology for obtaining pharmacokinetic data non-invasively is therefore required.

This thesis contains two examples of studies in which pharmacokinetic data have been collected, without causing considerable burden to participating patients. Patients undergoing an oral clonidine test have an intravenous cannula for growth hormone sampling in 30-minute intervals. Obtaining additional samples for determination of clonidine serum levels thus did not require additional venipunctures. By developing a sensitive assay, clonidine levels could be determined at up to 9 timepoints per patient without drawing an unacceptable volume of blood for pharmacokinetic analysis (Chapter 4). The dose of clonidine used in clinical practice today is still based on the original publication, which did demonstrate effectiveness of this dose, but did not include dose-finding (15), so obtaining serum levels of clonidine was considered useful in exploring whether lower dose ranges could be effective. It also provides data that can be used to establish oral bioavailability of the intravenous clonidine solution that we used.

The methylphenidate study in Chapter 6 demonstrates another approach that can be used in order to obtain pharmacokinetic data using minimally invasive methodology. By measuring methylphenidate concentrations in saliva, we were able to obtain methylphenidate levels without having to perform venipunctures. It is not very likely that we would have succeeded in performing this study had we included one or more venipunctures in the study protocol, as recruitment of participants would undoubtedly have been unsuccessful. By establishing the relationship of methylphenidate concentrations in blood and saliva in adult volunteers, we hope to be able to estimate the plasma levels of the ADHD patients in our study, which in turn could then be used to perform PK-PD modeling with the data.

OBTAINING PHARMACODYNAMIC DATA WITH MINIMAL BURDEN TO THE PATIENT

In addition to measurement of pharmacokinetic data, there is also a need for minimally invasive methodology for assessment of pharmacodynamics. Developmental changes do not only influence the pharmacokinetic parameters of a compound, but can also influence the biological response to a drug, for example by a different receptor density in the central nervous or cardiovascular system. Therefore, study of pharmacodynamic responses to a compound in relation to age and maturation are also needed and cannot simply be extrapolated from studies in adults.

As we have shown in Chapters 5 and 6 of this thesis, minimally invasive methods available from adult research can also be put to good use in pediatric research. Once it is established that such instruments can discriminate drug effects (like for example the body sway measurement used in Chapter 6), it can serve as a biomarker for drug effects, and be used in PK-PD modeling techniques, dose finding studies, or studies comparing immediate and controlled release preparations to provide some form of quantitative information on drug effect. Development of novel assessment methods can also prove to be a successful approach, as has been shown for example with the validation of the COMFORT behavior scale (16). This scale has been used in a range of studies investigating

sedatives and analgesics, and is becoming part of routine clinical care as an instrument to monitor the effectiveness of analgesia and/or sedation in hospitalized infants.

As we have demonstrated in Chapter 7, development of new measurement tools requires rigorous validation before application in practice. The device for bedside measurement of the activated partial thromboplastin time (APTT) that we evaluated turned out to overestimate the true underlying APTT value determined in the central lab.

ANALYSIS EFFICIENCY

Even more than in adult studies, methods increasing the yield of gathered materials and data are required to generate as much knowledge as possible from the obtained data. This can be done at several levels. For example, efforts must be made to increase sensitivity of pharmacokinetic assays. The Pharmacool study cited above uses blood samples of 200 microliters per timepoint for measurement of antibiotic plasma levels, thereby creating the opportunity of drawing multiple samples without reaching an unacceptable level of blood drawn (14). In general, drawing 3-5% of circulating volume is viewed as the allowed maximum in research involving neonates or children. In a preterm neonate of 1 kg, this will correspond to no more than 2.4 – 4 mL of blood that can be drawn for research purposes, so highly sensitive assays are indispensible.

A different level where the yield of obtained data can be increased, is the analysis phase. In pediatric studies, it is often much easier to obtain perhaps one pharmacokinetic sample per patient (for example combined with routine laboratory investigations for clinical monitoring) than obtaining 10 samples per patient. To establish a pharmacokinetic profile despite having only limited data for each patient, advanced analysis methods can be employed. In a so-called population approach, all available data (both pharmacokinetic and pharmacodynamic) can be entered into an integrated model. An approach that is frequently used is the program NONMEM (non-linear mixed effects modeling), which estimates the required pharmacokinetic parameters with a maximum likelihood approach (17). When designing a pharmocakinetic study, a trade-off needs to be made between the number of samples per patient and the amount of patients entering the study; sparse sampling requires a larger group of patients, while intensive sampling requires a smaller group of patients to generate a reliable pharmacokinetic profile.

Conclusion

In conclusion, there is a long way to go until truly adequately researched pharmacotherapy in pediatrics will be reached. There is a need for development of new formulations and new methodology to be used in the studies assessing new and existing compounds and formulations. Hopefully, the legislation that is now in place with regard to pediatric drug research will give an impulse to the infrastructure required to reach these goals. Funds invested by pharmaceutical companies will probably be aimed at research on novel compounds, as there is an obligation to study these new compounds in children under the current legislation. The rewards for the current efforts are excessive and moreover not spent on research infrastructure or new methodology. By creating more equality in the distribution of the additional profits earned by the pharmaceutical industry as a result of pediatric exclusivity, such funds can be redirected to a sustainable long-term research effort. At present, pediatric exclusivity is primarily an enormous (public) reward to pharmaceutical companies and their shareholders in return for fairly modest investments.

In order to also increase knowledge on existing and mostly off-patent compounds, public funds are needed to support this research. This has been recognized in the political arena, with large research budgets becoming available both in Europe (through the 7th Framework Programme) and in the United States (through the NIH) to support research on compounds

mentioned on the priority lists mentioned earlier. It is unlikely that funding of this research will originate from commercial parties, as a substantial investment will be required, and for a commercial company the revenue will not be sufficient to justify such investments. Once research is performed, governments must also ensure that this information reaches both the public and prescribers, by updating product information leaflets, and by maintaining up-to-date pediatric formularies available to prescribers, like the Children's edition of the British National Formulary in the United Kingdom, and the web-based Children's Drug Formulary in the Netherlands. As it is vital that this information is freely available and unbiased, these reference sources can only function properly under public funding.

1 Boots I, Sukhai RN, Klein RH, Holl RA, Wit JM, Cohen AF, et al. Stimulation programs for pediatric drug research--do children really benefit? Eur J Pediatr. 2007;166(8):849-55. DOI 10.1007/s00431-006-0381-z

2 Li JS, Eisenstein EL, Grabowski HG, Reid ED, Mangum B, Schulman KA, et al. Economic return of clinical trials performed under the pediatric exclusivity program. JAMA. 2007;297(5):480-8. DOI 10.1001/jama.297.5.480

3 Nelson RE, McAdam-Marx C, Evans ML, Ward R, Campbell B, Brixner D, et al. Patent extension policy for paediatric indications: an evaluation of the impact within three drug classes in a state Medicaid programme. Appl Health Econ Health Policy. 2011;9(3):171-81. DOI 10.2165/11539060-000000000-00000

4 Olski TM, Lampus SF, Gherarducci G, Saint Raymond A. Three years of paediatric regulation in the European Union. Eur J Clin Pharmacol. 2011;67(3):245-52. DOI 10.1007/s00228-011-0997-4

5 Milne CP, Bruss JB. The economics of pediatric formulation development for off-patent drugs. Clin Ther. 2008;30(11):2133-45. DOI 10.1016/j.clinthera.2008.11.019

6 Nahata MC. Lack of pediatric drug formulations. Pediatrics. 1999;104(3 Pt 2):607-9.

7 Whittaker A, Currie AE, Turner MA, Field DJ, Mulla H, Pandya HC. Toxic additives in medication for preterm infants. Arch Dis Child Fetal Neonatal Ed. 2009;94(4):F236-40. DOI 10.1136/adc.2008.146035

8 Allegaert K, Vanhaesebrouck S, Kulo A, Cosaert K, Verbesselt R, Debeer A, et al. Prospective assessment of short-term propylene glycol tolerance in neonates. Arch Dis Child. 2010;95(12):1054-8. DOI 10.1136/adc.2010.190330

9 Kulo A, de Hoon JN, Allegaert K. The propylene glycol research project to illustrate the feasibility and difficulties to study toxicokinetics in neonates. Int J Pharm. 2012. DOI 10.1016/j.ijpharm.2012.05.014

10 Koomen ER, Klein RH, Kweekel DM, Sukhai RN, Oostdijk W. Levothyroxine bij neonaten en kinderen met een voedingssonde [Levothyroxin administraiton to neonates and children with a nasogastric feeding tube]. Tijdschr Kindergeneeskd. 2012;80(1):17-9.

11 Allegaert K, Anderson BJ, Naulaers G, de Hoon J, Verbesselt R, Debeer A, et al. Intravenous paracetamol (propacetamol) pharmacokinetics in term and preterm neonates. Eur J Clin Pharmacol. 2004;60(3):191-7. DOI 10.1007/s00228-004-0756-x

12 Peeters MY, Prins SA, Knibbe CA, Dejongh J, Mathot RA, Warris C, et al. Pharmacokinetics and pharmacodynamics of midazolam and metabolites in nonventilated infants after craniofacial surgery. Anesthesiology. 2006;105(6):1135-46.

13 Peeters MY, Prins SA, Knibbe CA, DeJongh J, van Schaik RH, van Dijk M, et al. Propofol pharmacokinetics and pharmacodynamics for depth of sedation in nonventilated infants after major craniofacial surgery. Anesthesiology. 2006;104(3):466-74.

14 de Haan TR, Bijleveld YA, van der Lee JH, Groenendaal F, van den Broek MP, Rademaker CM, et al. Pharmacokinetics and pharmacodynamics of medication in asphyxiated newborns during controlled hypothermia. The PharmaCool multicenter study. BMC Pediatr. 2012;12:45. DOI 10.1186/1471-2431-12-45

15 Gil-Ad I, Topper E, Laron Z. Oral clonidine as a growth hormone stimulation test. Lancet. 1979;2(8137):278-9.

16 Ambuel B, Hamlett KW, Marx CM, Blumer JL. Assessing distress in pediatric intensive care environments: the COMFORT scale. J Pediatr Psychol. 1992;17(1):95-109.

17 De Cock RF, Piana C, Krekels EH, Danhof M, Allegaert K, Knibbe CA. The role of population PK-PD modelling in paediatric clinical research. Eur J Clin Pharmacol. 2011;67 Suppl 1:5-16. DOI 10.1007/s00228-009-0782-9

CHAPTER 9

Summary / Samenvatting

This thesis covers a variety of topics around the central theme of pharmacological research involving children, with a specific focus on the development of minimally invasive methodology that can be employed in future studies involving children. Children form a unique group within the area of pharmacological research and pharmacotherapy. The heterogeneity even within this group is large, covering the range of preterm neonates weighing 500 grams up to adolescents. Obviously, therapeutic needs change across this range, as among others disease epidemiology, drug disposition, pharmacodynamic response, and suitable drug formulations change with age. The same holds true for the design of drug trials involving children: where pharmacokinetics in adults can be studied simply by recruiting a number of healthy volunteers, such a study with a number of healthy toddlers is clearly not feasible and not acceptable. Therefore, approaches and new methodology are needed to circumvent these issues.

CHAPTER 1 of this thesis provides a background scope on the topic of pharmacological research involving children. Until recently, the pharmaceutical industry was not required to submit data concerning children to the competent authorities. This changed in the late 1990's (US) and in 2006 (Europe) when the authorities started requiring the industry to present data on the pediatric population when applying for a novel marketing authorization, and at the same time offering rewards for new research on already marketed compounds. This has led to an impulse for research infrastructure specifically focused on the pediatric age group. On the other hand it has led to industry exploiting the new regulation by placing the 'center of gravity' of research on their blockbuster drugs, thereby creating substantial additional revenue. This chapter also shortly comments on the issue of ethics in trials involving children. Non-therapeutic research is almost inevitable in the process of adequate drug research (e.g. dose-finding, toxicity, basic pharmacokinetics). In minors, non-therapeutic research may not entail more than minimal risk and/or burden. The interpretation of minimal risk and burden are a subject of intense debate. Finally, the first chapter contains a short overview of the contents of the thesis.

CHAPTER 2 evaluates the effects of the pediatric exclusivity provision under the American Food and Drug Administration Modernization Act (FDAMA), later followed by the Best Pharmaceuticals for Children Act. This pediatric exclusivity essentially provides a drug manufacturer 6 months of additional market exclusivity, once this manufacturer delivers results of research in the pediatric population that is satisfactory to the FDA. We have reviewed the types of studies that have been performed in response to this legislation, and for which compounds. We found that 135 entities were granted pediatric exclusivity between 1998 and 2006, involving research with at least 40,000 patients from the pediatric age group. The spectrum of compounds researched closely matched the adult utilization patterns rather than utilization patterns of children. In addition, the amount of blockbuster drugs granted pediatric exclusivity was considerable. We concluded that although the development of a research infrastructure is certainly a positive development, but have also drawn attention to the facts that the additional profits to drug companies might be viewed as disproportionally large, and that an increased focus should be put on actual therapeutic needs of children.

The availability of formulations suitable for children is not always obvious. In **CHAPTER 3**, Levothyroxin is covered as an example of problems associated with suitable formulations being unavailable. In the Netherlands, common practice is to administer levothyroxine in the form of crushed tablets administered with water. Where this is referred is commonly referred to as a solution, it turns out to be a suspension due to very poor solubility of levothyroxine in water. We have shown that administration through a nasogastric feeding tube leads to loss of considerable amounts of drug in the tube, probably due to non dissolved tablet

particles remaining in the nasogastric feeding tube. Also administration of crushed tablets with water on a spoon leads to uncertainty, as most of the drug will be in the bottom of the spoon and not dissolved in water. We have recommended use of a preparation commercially available in other European countries. In contrast to the suspension described above, this commercial product is a solution. We have shown that this solution can be administered through nasogastric feeding tubes without problems.

CHAPTER 4 is an example of a study collecting combined pharmacokinetic and pharmacodynamic data. We have studied clonidine, a centrally active antihypertensive drug, which has been demonstrated to elicit growth hormone release in the late 70's. It is used as a diagnostic tool in patients, including children, with a suspected growth hormone deficiency. The diagnostic procedure, involving administration of clonidine, is associated with several untoward effects such as hypotension, sedation and hypoglycemias. By combining the collection of pharmacokinetic data with pharmacodynamic data, we were able to perform a combined analysis of the two using non-linear mixed effect modeling, an advanced mathematical method working with likelihood models to predict the true relationship of drug concentrations versus observed effects. Based on our data, we have concluded that the clonidine concentrations reached are probably well above those needed for maximum intentional and unintentional effects. Ideally, one would want to predict a lower effective dose level using the PK-PD model, but the highly complex underlying pattern of spontaneous growth hormone release has made a solid prediction difficult. We have therefore concluded that a lower dose is likely to be sufficient, but prospective studies are needed to test this hypothesis and to establish an evidence-based lower dose level.

In **CHAPTER 5** and **6**, we have employed neurocognitive tasks form the Neurocart in children aged 8-12 years of age. The Neurocart is a battery of neurocognitive tasks frequently used and extensively validated at the Centre for Human Drug Research. In **CHAPTER 5** we report on a group of healthy children performing 3 consecutive runs of neurocognitive testing. We identified learning effects for a number of tasks, which should be considered in future trial design. We also evaluated how children tolerated participation in the study, and demonstrated that this type of study is well-tolerated by children from this age group. In **CHAPTER 6**, we used the same battery in children with attention-deficit hyperactivity disorder. We designed a placebo controlled, 2-way crossover trial in which participating children were tested with several runs of neurocognitive testing after administration of placebo and after administration of methylphenidate. Significant differences between the placebo and methylphenidate condition were identified for a number of tasks, and in one case (adaptive tracking) there was a clear suggestion of a relationship with (expected) plasma methylphenidate concentrations. The plasma methylphenidate concentrations were not measured during the study, but saliva samples were collected. These data will be used to predict plasma concentrations of the participating children, using pharmacokinetic modeling with data obtained from a study in adults, in whom paired plasma and saliva samples were taken.

A bedside device for measurement of the activated partial thromboplastin time (APTT) is evaluated in **CHAPTER 7**. The bedside requires only a drop of blood for assessment of the APTT, whereas conventional methods require a tube to be sent off to the central lab. This means that the bedside device is less invasive, as it requires a substantially smaller amount of blood. We demonstrate why thorough validation of new methodology such as this bedside device is necessary. Paired samples were taken from infants on heparin therapy, one of which was measured using the bedside device, the other sample analyzed using the central lab as 'gold standard'. It turned out that the bedside device led to substantial overestimation of the APTT, especially at higher APTT levels. We have therefore concluded that the bedside device should not be used to monitor heparin therapy

in infants. The findings in this chapter should also be seen as a generic advice to thoroughly evaluate novel methodology.

The thesis concludes with a general discussion (**CHAPTER 8**). Apart from shortly summarizing the contents of the thesis, we have once again drawn attention to the financial aspects of legislation regarding drug research involving children. The current list of top 10 selling drugs in the United States contains 8 compounds for which pediatric exclusivity was granted. The nature of these drugs (including statins, antipsychotics and anticoagulant) reaffirms our prior statement that there is a mismatch of research performed under the pediatric exclusivity provisions with actual therapeutic needs. To address the true therapeutic needs, public funding will be needed (through the 7th Framework Programme in the EU and the NIH in the US), as there is no prospect of large revenue in this category of drug compounds. Once such research is performed, governments also have a responsibility to make the obtained information accessible to the public and prescribers. In the Netherlands, the open access and web-based Children's Drug Formulary is an excellent example of how newly obtained knowledge can be disseminated effectively. This example also illustrates the need for public funding: open access of unbiased information would be hard to achieve without support from public funds.

Samenvatting

In dit proefschrift wordt een aantal onderwerpen belicht rondom klinisch-farmacologisch onderzoek waarbij kinderen betrokken zijn. Hierbij ligt de nadruk op de ontwikkeling van minimaal invasieve meetinstrumenten, die in toekomstige studies met kinderen gebruikt zouden kunnen worden. Binnen zowel het klinisch farmacologisch onderzoek alsook de farmacotherapie vormen kinderen een unieke groep. De heterogeniteit is zelfs binnen deze groep al groot, met een spectrum dat loopt van premature van 500 gram tot jong-volwassenen. Binnen dit spectrum verschuiven therapeutische behoeften, farmacokinetische parameters, farmacodynamische respons, en geschikte toedieningsvormen met de leeftijd. Ook bij het opzetten van geneesmiddelenonderzoek moet rekening gehouden worden met de bijzondere positie van kinderen: waar bij volwassenen een farmacokinetiek-studie met gezonde vrijwilligers zonder meer acceptabel is, is een dergelijke studie met gezonde kleuters welhaast ondenkbaar. Daarom zijn vernieuwende benaderingen en nieuwe meetmethoden nodig om toch betrouwbaar en zinvol onderzoek te kunnen verrichten in deze bijzondere groep.

In **HOOFDSTUK 1** wordt enige achtergrondinformatie gegeven met betrekking tot klinisch farmacologisch onderzoek in de kindergeneeskundige populatie. De farmaceutische industrie was tot enkele jaren terug niet verplicht om bij de autoriteiten gegevens aan te leveren die specifiek betrekking hadden op kinderen. Dit veranderde eind jaren '90 (VS) en in 2006 (Europa). Sindsdien verlangen de instanties ook gegevens over kinderen voordat nieuwe geneesmiddelen toegelaten worden tot de markt, en worden farmaceuten beloond als zij dergelijke data aanleveren voor middelen die reeds op de markt zijn. Dit heeft geleid tot een forse impuls in de ontwikkeling van onderzoeksinfrastructuur die specifiek gericht is op de uitvoering van geneesmiddelenonderzoek met kinderen. Anderzijds

heeft de geneesmiddelenindustrie deze regelgeving ook uitgebuit voor eigen gewin, door het zwaartepunt van haar inspanningen te leggen bij die middelen die voor de industrie zeer winstgevend zijn. Binnen hetzelfde hoofdstuk wordt ook kort stilgestaan bij aspecten van de ethiek van geneesmiddelenonderzoek bij kinderen. Het is welhaast onmogelijk om de relevante gegevens voor een middel te verzamelen, zonder dat hiervoor ook niet-therapeutisch onderzoek plaatsvindt. Bij minderjarigen is dergelijk onderzoek alleen toegestaan als de risico's en belasting minimaal zijn. De interpretatie van het criterium 'minimaal' is momenteel onderwerp van discussie. Tot slot wordt in hoofdstuk 1 een kort overzicht gegeven van de verdere inhoud van het proefschrift.

In **HOOFDSTUK 2** worden de effecten van de Amerikaanse regelgeving (Food and Drug Administration Modernization Act, Best Pharmaceuticals for Children Act) geëvalueerd. De kern van deze regelgeving bestaat uit een verlenging van het verleende patent voor de duur van 6 maanden, als over het betreffende geneesmiddel aan de Amerikaanse autoriteiten gegevens worden aangeleverd met betrekking tot de kindergeneeskundige populatie. We hebben bekeken voor welke middelen er naar aanleiding van deze regelgeving onderzoek werd verricht. Tussen 1998 en 2006 werd voor 135 geneesmiddelen het patent met 6 maanden verlengd, na onderzoek waarbij zeker 40.000 kinderen betrokken waren. Het spectrum van middelen dat onderzocht werd, kwam sterk overeen met de middelen die door volwassenen gebruikt worden. Daarnaast bevond zich onder de onderzochte middelen een opvallend groot aantal 'blockbusters'. Wij concluderen in dit hoofdstuk dat er met de opkomst van een op kinderen toegeruste onderzoeksinfrastructuur een zeer positieve ontwikkeling gaande is, maar dat de beloning die de farmaceutische industrie hiervoor ontvangt in de vorm van verlenging van het patent in sommige gevallen leidt tot disproportioneel grote winsten. Tevens moet er naar onze mening beter gekeken worden naar de middelen die voor kinderen zinvol zijn.

De beschikbaarheid van voor kinderen geschikte toedieningsvormen is niet altijd vanzelfsprekend. In **HOOFDSTUK 3** wordt aan de hand van het geneesmiddel levothyroxine geïllustreerd hoe het ontbreken van een geschikte toedieningsvorm tot problemen kan leiden. In Nederland is het (bij toediening aan kinderen) gangbaar om levothyroxine tabletten te vergruizen en met water toe te dienen. Waar algemeen wordt aangenomen dat zo een oplossing wordt bereid, blijkt het te gaan om een suspensie ten gevolge van zeer slechte oplosbaarheid van levothyroxine in water. We hebben aangetoond dat, waarschijnlijk ten gevolge van het achterblijven van tabletresten, toediening van deze suspensie via een maagsonde kan leiden tot aanzienlijk verlies van geneesmiddel in de sonde. Ook toediening van vergruisde tabletten op een lepel met water leidt tot grote onzekerheid in de toediening, aangezien het geneesmiddel zich voor het overgrote deel bevindt op de bodem van de lepel, en niet in oplossing is gekomen. Naar aanleiding van dit onderzoek bevelen wij aan om over te gaan tot gebruik van een commercieel preparaat dat elders in Europa op de markt te verkrijgen is. In tegenstelling tot de bereide suspensie zoals hierboven beschreven, betreft dit preparaat een oplossing. We hebben aangetoond dat dit commerciële product wel zonder problemen gebruikt kan worden met een maagsonde.

HOOFDSTUK 4 bevat een voorbeeld van geneesmiddelenonderzoek bij kinderen, waarbij zowel farmacokinetische als farmacodynamische data verzameld werden. Wij bestudeerden clonidine, een centraal aangrijpend antihypertensivum, waarvan sinds de jaren '70 tevens bekend is dat toediening van dit middel leidt tot afgifte van groeihormoon. Dit middel wordt, ook bij kinderen, gebruikt als diagnosticum bij verdenking op een groeihormoondeficiëntie. De diagnostische procedure, waarbij clonidine oraal wordt toegediend, is geassocieerd met meerdere bijwerkingen waaronder hypotensie, sedatie en hypoglycaemieën. Door bij kinderen die deze procedure ondergingen zowel farmacokinetische als farmacodynamische data te verzamelen, konden wij een gecombineerde analyse verrichten

met behulp van 'non-linear mixed effect modeling', een geavanceerde mathematische methode waarbij met kansberekening wordt gepoogd de onderliggende 'ware' relatie tussen geneesmiddelspiegel en het waargenomen effect te voorspellen. Op basis van onze data hebben wij geconcludeerd dat de bereikte clonidine spiegels waarschijnlijk veel hoger liggen dan nodig om maximale (bij-)effecten te bereiken. Idealiter zou men met behulp van het berekende model willen komen tot de voorspelling van een lagere effectieve dosis. Door een uitermate complex onderliggend patroon van groeihormoon-afgifte bleek dit moeilijk te voorspellen. We hebben ons derhalve moeten beperken tot de conclusie dat een lagere dosering zeer waarschijnlijk ook effectief zal zijn, maar aanvullend prospectief onderzoek zal nodig zijn om deze hypothese te testen.

In **HOOFDSTUK 5** en **6** laten wij zien hoe de Neurocart, een bij het Centre for Human Drug Research uitvoerig gevalideerde batterij met neurocognitieve tests, ook gebruikt kan worden bij kinderen in de leeftijd van 8-12 jaar. In hoofdstuk 5 rapporteren wij de testresultaten van een groep gezonde kinderen die de testbatterij in drie opeenvolgende sessies uitvoeren. Bij een aantal van de tests zagen we leereffecten, waarmee in het design van toekomstige studies rekening moet worden gehouden. Ook evalueerden wij in welke mate deelname aan dergelijk onderzoek voor kinderen belastend was, en toonden aan dat dit type onderzoek door kinderen zeer goed geaccepteerd wordt. In hoofdstuk 6 werd dezelfde testbatterij gebruikt in een groep kinderen met ADHD. In een placebo-gecontroleerd, 2-weg crossover onderzoek werden kinderen onderzocht na toediening van placebo en na toediening van methylfenidaat. Bij verschillende tests werden statistisch significante verschillen waargenomen tussen testresultaten onder placebo-conditie en onder methylfenidaat-conditie. Bij een van de tests leek er zelfs sprake te zijn van een relatie met de onderliggende verwachte plasmaconcentratie. De plasma concentraties werden tijdens dit onderzoek niet gemeten. Wel werden speekselmonsters afgenomen, zodat in de toekomst de onderliggende plasmaconcentraties met modelleertechnieken voorspeld kunnen worden. Hiervoor dient nog wel aanvullend onderzoek plaats te vinden bij gezonde volwassenen, om de relatie tussen plasma- en speekselconcentraties van methylfenidaat vast te stellen.

In **HOOFDSTUK 7** wordt een 'bedside test' voor de bepaling van de APTT bij kinderen geëvalueerd. Waar deze bedside test aan een druppel bloed genoeg heeft, moet voor de conventionele bepaling een buisje bloed naar het centrale ziekenhuis laboratorium verzonden worden. Dit betekent dat de bedside methode minder invasief is, aangezien er een beduidend kleinere hoeveelheid bloed voor nodig is. We laten zien waarom grondige validatie van dergelijke methoden nodig is: het blijkt dat simultaan afgenomen bepalingen bij geheparinieerde jonge kinderen op het bedside apparaat niet overeenkomen met de 'gouden standaard' in het ziekenhuislaboratorium. Vooral bij hogere APTT waarden was er bij het bedside apparaat sprake van substantiële overschatting van de APTT. Wij hebben op basis van dit onderzoek geconcludeerd dat het apparaat niet geschikt is voor het monitoren van heparine therapie bij jonge kinderen. De bevindingen in dit hoofdstuk moeten ook gezien worden als een generieke aanbeveling om nieuwe methodologie grondig te evalueren.

Het proefschrift wordt afgesloten met een algemene discussie (**HOOFDSTUK 8**). Naast een korte samenvatting van de inhoud van het proefschrift, wordt nogmaals de aandacht gevestigd op de financiële repercussies van de zowel in de VS als Europa geïmplementeerde wetgeving. Van de huidige top-10 van best verkopende geneesmiddelen in de Verenigde Staten zijn er 8 middelen die onder de wetgeving een verlenging van het patent hebben ontvangen. De aard van de betreffende geneesmiddelen (statines, antipsychotica en anticoagulantia) onderstreept nogmaals onze eerdere bewering dat er sprake is van een mismatch tussen therapeutische behoeften bij kinderen en het daadwerkelijk uitgevoerde onderzoek. Om ook deze ware therapeutische behoeftes te adresseren, zal aanvullende

financiering nodig zijn (bijvoorbeeld via het 7e Raamwerk Programma in de EU, en via het National Institute of Health in de Verenigde Staten). Dit heeft er niet in de laatste plaats mee te maken, dat de groepen geneesmiddelen die voor kinderen van belang zijn vaak niet commercieel interessant zijn. Als eenmaal onderzoek verricht is, ligt er vervolgens ook een verantwoordelijkheid voor overheden om de verkregen informatie toegankelijk te maken voor zowel het publiek als voorschrijvers. In Nederland is de website kinderformularium.nl een uitstekend voorbeeld van effectieve ontsluiting van de beschikbare gegevens. Dit voorbeeld illustreert tegelijkertijd het belang van ondersteuning met publieke middelen. Vrij toegankelijke en onbevooroordeelde informatiekanalen zouden zonder dergelijke ondersteuning moeilijk te realiseren zijn.

CURRICULUM VITAE

Richard Klein was born on the 26th of January 1981 in Luxembourg. He obtained the European Baccalaureate from the European School (Luxembourg) in 1998, and subsequently moved to Leiden to commence his medical education at the Leiden University Medical Center. After obtaining his medical degree in 2004, he worked as a resident (ANIOS) at the Langeland Ziekenhuis in Zoetermeer in 2005. In January 2006 he returned to Leiden as research physician at the Centre for Human Drug Research, a position he held until December 2008. Most of the work described in this thesis was performed within this period. He has started his residency in Pediatrics (AIOS) in January 2009 at the Groene Hart Ziekenhuis in Gouda, followed by the Leiden University Medical Centre from July 2010. He hopes to obtain his registration both as pediatrician and as clinical pharmacologist in the latter half of 2013.

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Lieve Heike. Wat is de rol van 'de partner van' in een traject als dit belangrijk. Zonder jouw steun en geduld was dit boekje er gewoon niet gekomen. Dit wordt het zoveelste life-event van het afgelopen jaar, en ik verheug me nu al op de life-events die we samen (met onze grote trots Jelte) nog gaan meemaken.

LIST OF PUBLICATIONS

1 Leijser LM, Klein RH, Veen S, Liauw L, Van Wezel-Meijler G. Hyperechogenicity of the thalamus and basal ganglia in very preterm infants: radiological findings and short-term neurological outcome. Neuropediatrics. 2004; 35(5): 283-9.

2 Boots I, Sukhai RN, Klein RH, Holl RA, Wit JM, Cohen AF, Burggraaf J. Stimulation programs for pediatric drug research--do children really benefit? European Journal of Pediatrics. 2007; 166(8): 849-55.

3 Koomen ER, Klein RH, Kweekel D, Sukhai RN, Oostdijk W. Levothyroxine bij neonaten en kinderen met een voedingssonde [Levothyroxine for neonates and children with a nasogastric feeding tube]. Tijdschr Kindergeneeskd. 2012; 80(1): 17-9.

4 Klein RH, van der Vorst MMJ, de Wilde RBP, Hogenbirk K, de Kam ML, Burggraaf J. Evaluation of a bedside device to assess the activated partial thromboplastin time for heparin monitoring in infants. Blood Coag Fibrinolysis. in press.