Challenges in the treatment of HIV-1 infected children with highly active antiretroviral therapy

Cover: © Willem van Scheijndel: Moeders met kinderen
"Ouderliefde tref je overal ter wereld aan: deze onbeschrijfelijke oerdrang om je kind te beschermen tegen al het kwaad in de wereld en de behoefte dicht bij elkaar te blijven op moeilijke momenten."

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Challenges in the treatment of HIV-1 infected children with highly active antiretroviral therapy

Uitdagingen in de behandeling van met HIV-1-geïnfecteerde kinderen

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Chapter 1

Introduction

Nobody could have predicted the tremendous implications of the headline in The New York Times of July 5, 1981: "Rare cancer in homosexuals". This article contained the first description of the disease "Acquired Immune Deficiency Syndrome" (AIDS) and marked the beginning of a pandemic by the Human Immunodeficiency Virus (HIV). (1) Three years after the first adults with AIDS were identified, similar disease features were reported in children. (2, 3) In general, HIV infection progresses more rapidly in children than in adults. This progression is associated with a higher viral burden, a more rapid depletion of CD4+ T-cell lymphocytes and impaired growth characteristics. (4-8) HIV infected children may be divided in three groups on the basis of disease progression: "rapid progressors" (±20%), "intermediate progressors" (±60%) and "slow progressors" (±20%). (9-12) The first group shows a rapid decrease in CD4+ T-cells and develops AIDS (CDC classification C (13)) within the first two years of life. In "intermediate progressors" a more gradual decrease in CD4+ T-cell counts is observed. These children don't have symptoms of serious immunosuppression until the age of 7-8 years. The "slow progressors" are asymptomatic at the age of 8 years and have a normal or slightly decreased CD4+ T-cell count. Symptoms associated with HIV infection in infants and children are dependent on the extent of immunosuppression. The Centers for Disease Control and Prevention have developed a pediatric classification system based on the seriousness of symptoms. (13) In children with intermediate and slow progression rates an asymptomatic medical history may be present possibly in combination with a slightly increased incidence of bacterial infections and lymphadenopathy. Pneumocystis carinii pneumonia (PCP) was the most frequently occurring opportunistic infection in HIV infected children during the initial period when HIV exposed and HIV infected children in Europe and the USA did not routinely receive PCP-prophylaxis. (14)

In the early 80's most of the HIV infected children acquired infection by means of blood transfusion. With the introduction of HIV specific antibody tests in 1985, testing of donor blood became possible thus preventing this mode of transmission. At that time the HIV-epidemic in the Western world was mainly spread through the homosexual population and relatively small numbers of HIV infected children were reported. However, since the early 90's the numbers of children with perinatally acquired HIV infection increased rapidly as a result of the spread of disease in the heterosexual population. (15) Mother-to-child transmission became the most important mode of infection in children not only in developing countries, but also in the Western world. In Western Europe the increase in HIV-1 infected children is mainly caused by migration of HIV infected women and children from countries with a HIV epidemic (sub-Saharan Africa and The Carribean). (16)

The HIV/AIDS epidemic has an enormous impact on the health and well-being of millions of children and adults all over the world, but especially in Africa and Asia. Ninety percent of the HIV infected people live in these parts of the world (Figure 1). At this moment women, of childbearing age constitute nearly half of the 40 million adults living with HIV/AIDS. The risk of mother-to-child transmission of HIV can nowadays be easily reduced. Nevertheless, mother-to-child transmission during pregnancy, delivery and breast-feeding is still accounting for approximately 10% of the 16000 new infections that occur each day worldwide. (15)

In the first study (ACTG076) to prevent mother-to-child transmission in 1994, administration of zidovudine to pregnant women and to their children during the first 6 weeks of their lives resulted in a reduction of the transmission rates of 67%. A transmission rate of 22.6%

was found in the placebo group whereas in the group that received zidovudine a transmission rate of 7.6% was observed. (17) In the Western World transmission rates may be reduced to almost 0% by means of treatment of the mother with HAART and by the administration of antiretroviral drugs to their children. An elective cesarean section prevents transmission during birth in mothers who have a detectable viral load at the time of birth. (18, 19) In developing countries where HAART is frequently not available it has become possible to reduce the risk of mother-to-child transmission by cheap medical interventions. Although the ACTG076 study provided a relatively easy way to reduce the risk of mother-to-child transmission, easier and less costly alternatives using short-course, single-drug regimens of zidovudine or nevirapine became available in 1999. (20, 21) In these studies the transmission rates were reduced with 50%.

The millions of infected pregnant women give birth to many children that are not infected, but affected by the HIV epidemic, because of ongoing disease in their parents. Living in a family with HIV infected parents and siblings that are infected is a social and psychological disaster for these children who will eventually end up as orphans. Since the beginning of the HIV epidemic a total of 13.2 million children < 15 years have thus become orphans. (15)

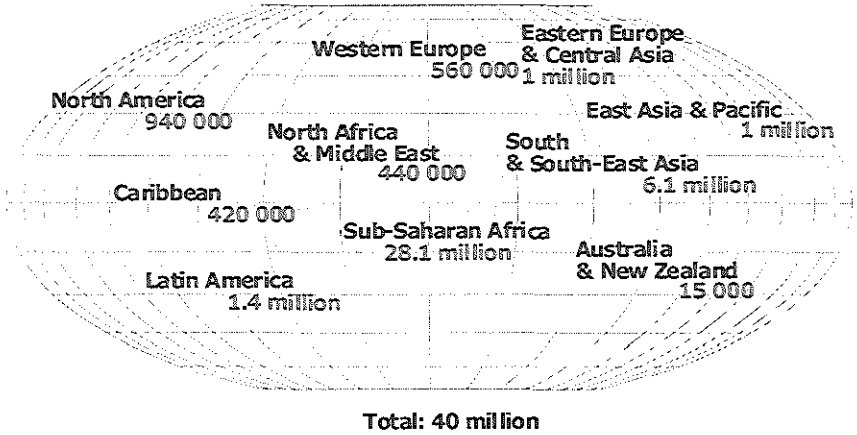

Figure 1 Adults and children estimated to be living with HIV/AIDS as of end 2001

In The Netherlands the University Children's Hospitals in Amsterdam, Rotterdam and Utrecht were confronted during the 1990's with gradually increasing numbers of HIV-1 infected children from women in which the virus was transmitted by heterosexual contact. (22) Prevention programs resulted in a reduction of the transmission rates of HIV in homosexuals. Yet, simultaneously an increase in the number of heterosexually infected women from countries with a generalized HIV-epidemic was observed. (22)

The prevalence of HIV infection in pregnant women in Amsterdam increased from 0.2% in the early 90's to 0.8% in 1999. Eighty percent of these women originated from countries other than The

Netherlands. (23) This also influenced the data on HIV infected children. During the period 1998-2000 in 83% of the HIV infected children, the parents originated from countries with a generalized HIV epidemic. HIV infections in children in The Netherlands are almost solely caused by type 1. Thusfar only one child with an HIV-2 infection has been identified. (24) This may be due to the smaller mother-to-child transmission rates of HIV-2 as well as to the lower prevalence of HIV-2 in comparison to HIV-1. (25, 26)

The increasing number of HIV-1 infected children in The Netherlands urged a group of Dutch pediatricians, specialized in infectious diseases in 1997 to create a multidisciplinary Study Group for HIV-1 infected children in order to be able to provide optimal care for HIV-infected children. This team included experts from the fields of Pediatric Infectious Diseases, Pharmacology, Immunology and Virology. Since data on the treatment of HIV infected children were only scarcely available, it became clear that optimal care could only be provided in the context of a structured scientific approach to the diagnosis and treatment in which new developments from the different fields of expertise especially from frontline knowledge in adult infectious diseases could be rapidly transferred to the pediatric field. A study protocol on the treatment of HIV-1 infected children with indinavir, zidovudine and lamivudine was written on the basis of the excellent preliminary clinical, virological and immunological results in adults treated with this regimen. The relative immaturity of the immune system, differences in pharmacokinetics and pharmacodynamics of antiviral drugs and specific issues concerning adherence to therapy, complicated a simple extrapolation from results in adults to those in children. Therefore the study protocol contained strict clinical, virological, immunological and pharmacological monitoring criteria. This thesis contains the results of these studies and provides an overall insight in the current state of the art on the care, the epidemiology, the diagnosis and the treatment of HIV infected children.

It is unacceptable that the improving possibilities to prevent mother-to-child transmission and to treat HIV infection are not yet available in developing countries and not even in second world countries (for example Romania). In 2001, pharmaceutical companies were urged to reduce the costs of antiretroviral drugs. However, to provide care for HIV infected children, not only antiretroviral therapy is needed, but also improvements in the infrastructure for health care, better health care education and a broader availability and use of vaccines against other infectious diseases are necessary. Without appropriate health care the administration of antiretroviral therapy will rapidly result in increasing numbers of non-compliant children, the selection of resistant virus mutants and in the subsequent failure of therapy. Since in The Netherlands 86% of newly detected HIV-1 infected children originate from a country with a generalized HIV epidemic, a spread of resistant virus mutants in these countries could subsequently result in an increase of children infected with resistant virus mutants in The Netherlands. Years after antiretroviral medication became available in the Western world, finally the United Nations, the governments of many countries and the multinational pharmaceutical companies have taken a first step towards the institution of a Global Health Fund to improve the health care, education and prevention all over the world. Hopefully, these improvements are in time to prevent a broad spread of resistant virus mutants.

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Outline of this thesis

Chapter 2

Outline of this thesis

Part one contains epidemiological studies on HIV-1 infection in children in The Netherlands. Since 1995 a prospective surveillance of all children diagnosed with HIV infection or exposed to HIV has been performed in The Netherlands. Pediatricians monthly report these children to the Dutch Pediatric Surveillance Unit. Until 1997 the Department of Pediatrics of the Wilhelmina Children's Hospital (Utrecht) was responsible for the distribution of questionnaires and the data analysis. Since 1998 this registration has been performed by the Department of Pediatrics, division of Pediatric Infectious Diseases and Immunology of the Sophia Children's Hospital (Rotterdam). In **chapter 3** we report the results of the program to prevent transmission of HIV by means of antiretroviral therapy to children of HIV infected mothers in The Netherlands in the period 1995-2000. In **chapter 4** we present the data on the cumulative number of children living in The Netherlands who were diagnosed with HIV-1 infection, on their mode of transmission, their risk factors and their clinical features from the start of the AIDS epidemic in the 1980's until 2000.

In part two several studies dealing with the pharmacokinetics and pharmacodynamics of antiretroviral agents in HIV-1 infected children are presented. In **chapter 5** we present a study on the pharmacokinetics and pharmacodynamics of the protease inhibitor indinavir in HIV-1 infected children. Since indinavir was administered three times a day in a fasting state, a simplified medication regimen was urgently needed. In **chapter 6** we therefore present data on the pharmacokinetics of a combination of indinavir with low-dose ritonavir, which was given in a twice-daily regimen without food restrictions. This study forms the basis for future studies with simplified twice-daily regimens in children. In **chapter 7** we questioned whether therapeutic drug monitoring (TDM) is a useful tool to assess compliance in HIV-1 infected children. The data generated by TDM were correlated with the virological outcome.

Part three contains the clinical, virological and immunological impact of highly active antiretroviral therapy (HAART) on HIV-1 infected children. Since the introduction of protease inhibitors in 1996, combination therapies including two reverse transcriptase inhibitors and a protease inhibitor have rapidly become standard therapy for HIV-1 infected adults. The data on efficacy of these combinations in children with HIV-1 infection are limited, and are predominantly derived from studies in which only small numbers of children are included. **Chapter 8** provides an overview of the clinical studies using HAART in children and seeks to improve the understanding of factors, which may contribute to success or failure of HAART in children. In **chapter 9** we report the clinical and virological responses to combination treatment with indinavir, zidovudine and lamivudine in HIV-1 infected children until 24 weeks of follow up. In **chapter 10** the long-term clinical, virological and immunological responses to HAART (containing indinavir or nelfinavir) are presented. Since data on long-term T-cell dynamics in HIV-1-infected children on HAART were not available, it was still unclear to what extent the number of CD4+ T-cells of HIV-1-infected children could be normalized. In **chapter 11** we evaluated the extent of the long-term immune reconstitution in HIV-1-infected children who were treated with HAART during a period of 96 weeks. We analyzed changes in the number of CD4+ T-cells, CD4+ T-cell naive and memory subsets and CD8+ T-cell counts and compared those with the normal values that we previously reported for different age groups. In

addition to the quantitative analyses, the T-cell function after stimulation with CD3 mAb plus CD28 mAb was analysed.

Dysregulation of growth is a common feature of HIV-1 infection in children. Since growth seems to be one of the most sensitive indicators of disease progression, growth after the initiation of HAART is an important parameter. Growth characteristics of HIV-1 infected children after the initiation of HAART and the association between growth parameters and virological and immunological response to HAART were studied. These data are reported in **chapter 12**. The etiology of the HIV-1 related growth dysregulation is complex. Abnormal function of the thyroid gland, the somatomedine axis, the lipid metabolism and abnormal resting energy expenditure may also contribute to diminished growth. We therefore studied the endocrinological and immunological mechanisms underlying the recovery of growth in HIV-1 infected children treated with HAART and present the results of these studies in **chapter 13**.

Antiretroviral drugs such as indinavir need to be continued for many years thus underscoring the importance of a careful surveillance for long-term toxicity. Studies on the side effects of HAART are presented in **part four. Chapters 14 and 15** describe the occurrence of urinalysis abnormalities and nephrotoxicity in HIV-1 infected children treated with indinavir and study the relation between these side-effects and pharmacological parameters. **Chapter 16** contains a study on changes in the lipid and glucose metabolism after the initiation of HAART in HIV-1 infected children.

Chapters 17 and 18 summarize the previous studies and bring the data in perspective in English and Dutch language, respectively. In **Chapter 19** we briefly summarize our conclusions and we present our ideas concerning the future directions of care and research in children with HIV/AIDS.

Part I

Epidemiological studies

Reduction of mother-to-child transmission of HIV-1 infection in The Netherlands during the period 1995-2000

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Chapter 3

Abstract

Objectives: 1. Registration of the number of children born to HIV infected mothers diagnosed before the delivery and 2. Analysis of the efficacy of the prevention of mother-to-child transmission of HIV-1 in the period 1995 to 1999. Design: Prospective registration from January 1995 to December 1999. *Methods:* Dutch paediatricians reported monthly HIV-1 exposed children to the Dutch Paediatric Surveillance Unit. All reports were followed up with standard questionnaires. An additional retrospective study was performed because of incomplete registration. *Results:* We observed an increase in the number of children known to be exposed to HIV from 5 to 25 in 1999. At the same time, the percentage of HIV-1 infected children decreased from 20% to 4%. The number of pregnant HIV-1 infected women using highly active antiretroviral therapy increased during the studyperiod from 0% to 70%. Antiretroviral therapy was administered to 92% of the HIV-1 exposed children . Only 2% of the children received breastfeeding. *Conclusion:* Despite an increase in the number of children known to be exposed to HIV, a decrease in the percentage of HIV infected children was observed. Only 4% of the children born in 1999 and known to be exposed to HIV was infected. We conclude that the Dutch guidelines to prevent mother-to-child transmission of HIV-1 infection are well implemented and efficacious.

Samenvatting

Doel: 1. Registratie van het aantal aan HIV geëxposeerde kinderen waarvan van de moeder ten tijde van de zwangerschap bekend was dat ze HIV geïnfecteerd was en 2. Een evaluatie van het beleid dat in de periode 1995 tot en met 1999 gevoerd is om overdracht van HIV-1 infecties van moeder naar kind te voorkomen. *Opzet:* Prospectieve registratie in de periode 1 januari 1995 tot 31 december 1999. *Methode:* Nederlandse kinderartsen meldden maandelijks via het Nederlands Signaleringscentrum Kindergeneeskunde ieder aan HIV geëxposeerd kind. De meldingen werden gevolgd door toezending van vragenlijsten. Vanwege gebleken onvolledige melding werd een aanvullend retrospectief onderzoek verricht. *Resultaten:* In de periode 1995-2000 nam het aantal aan HIV-1 geëxposeerde kinderen waarvan van de moeder ten tijde van de zwangerschap bekend was dat ze HIV geïnfecteerd was toe van 5 naar 25 per jaar. Het percentage HIV-geïnfecteerde kinderen daalde van 20% naar 4%. In de onderzoeksperiode steeg het gebruik van krachtige combinatie therapie door zwangere HIV-1 geïnfecteerde vrouwen van 0% naar 70%. Van de aan HIV geëxposeerde kinderen kreeg 92% antiretrovirale therapie. Slechts 2% van de kinderen kreeg borstvoeding. *Conclusie:* Ondanks een stijging van het aantal bekende aan HIV-1 geëxposeerde kinderen werd een daling gevonden van het percentage met HIV-1 geïnfecteerde kinderen. In 1999 werd slechts 4% van de aan HIV-1 geëxposeerde kinderen geïnfecteerd. Wij concluderen dat de in Nederland genomen maatregelen bij HIV positieve zwangere vrouwen en hun kinderen om overdracht van infectie te voorkomen effectief zijn.

Inleiding

De kans op verticale transmissie van humane immuundeficiëntie-virus (HIV) van moeder naar kind bedraagt zonder maatregelen 15-30% (1-4). Deze kans kan tegenwoordig tot minder dan 2% gereduceerd worden. In 1994 werd voor het eerst gerapporteerd dat de overdracht van HIV van

moeder naar kind significant gereduceerd kan worden (van 25,5% naar 8,3%) door toediening van antiretrovirale medicatie aan de zwangere vrouw en haar kind. (5, 6) Een electieve sectio caesarea bleek het transmissiepercentage verder omlaag te kunnen brengen naar 1-2%. (7, 8) Hetzelfde lage transmissiepercentage wordt waargenomen bij de pasgeboren kinderen van zwangere vrouwen bij wie door behandeling met krachtige antiretrovirale combinatietherapie tijdens de partus geen virus in het bloed meer aantoonbaar was. (9)

In Nederland worden HIV geïnfecteerde zwangeren in daartoe gespecialiseerde centra volgens richtlijnen van de Nederlandse Vereniging van Aids Behandelaren (NVAB) behandeld met antiretrovirale combinatietherapie. (10) Afhankelijk van de plasma virus concentratie vlak voor de partus wordt het kind vaginaal (< 500 kopieën/ml plasma) dan wel door middel van een sectio caesarea (overwegen bij viral load tussen 500 en 1000 kopieën/ml en in alle gevallen bij > 1000 kopieën/ml plasma) geboren. De neonaat wordt vervolgens gedurende 4 weken behandeld met zidovudine en lamivudine. Indien bij moeder tijdens de zwangerschap sprake is van onvoldoende reactie op de therapie (HIV-1 RNA >400 kopieën/ml) of als moeder niet behandeld is en de HIV-1 RNA verhoogd of onbekend is, wordt in overleg met een kinder-aidsbehandelcentrum afgeweken van de standaard duotherapie. (10)

Sinds 1995 is via het Nederlands Signalerings-Centrum Kindergeneeskunde (NSCK), aanvankelijk tezamen met de afdeling kindergeneeskunde van het Universitair Medisch Centrum Utrecht/Wilhelmina Kinderziekenhuis en vanaf 1998 in samenwerking met het Academisch Ziekenhuis Rotterdam/Sophia Kinderziekenhuis, het aantal aan HIV-1 geëxposeerde kinderen prospectief geregistreerd. In dit artikel worden de resultaten van deze registratie beschreven.

Patiënten en methoden

Met ingang van 1 januari 1995 melden praktiserende kinderartsen maandelijks elke nieuwe patiënt (0-18 jaar) met tenminste één van de volgende inclusiecriteria bij het NSCK: bewezen aids, positieve HIV serologie, positieve viruskweek, positieve polymerasekettingreactie (PCR) op HIV-RNA, positieve p24 antigeenbepaling of een pasgeborene van een HIV positieve moeder met een nog onbekende HIV serostatus. Na melding van een HIV geïnfecteerd kind of een aan HIV geëxposeerd kind wordt de betreffende kinderarts verzocht een vragenlijst in te vullen met vragen over klinische en sociale kenmerken van het gemelde kind.

De melding van aan HIV geëxposeerde kinderen bleek echter onvolledig te zijn. Daarom werd in 2000 een retrospectief onderzoek verricht waarbij de gegevens van alle kinderen van HIV geïnfecteerde vrouwen, geboren tussen 1 januari 1995 en 31 december 1999, via kinderartsen in de elf door de Gezondheidsraad aangewezen aids behandelcentra en de vier daaraan geaffilieerde ziekenhuizen achterhaald werden. Van de gemelde en opgespoorde aan HIV geëxposeerde kinderen werd met behulp van een follow-up vragenlijst de HIV-status op de leeftijd van 18 maanden (negatieve HIV serologie op de leeftijd van 18 maanden geldt als definitieve uitsluiting van een HIV infectie) vastgesteld. Alle gegevens werden anoniem verwerkt.

In dit artikel worden de resultaten beschreven van de registratie van aan HIV geëxposeerde kinderen van moeders met een voor de partus vastgestelde HIV-infectie.

De HIV-status van de aan HIV geëxposeerde kinderen werd als volgt gedefinieerd:

HIV geïnfecteerd bij:

- Aanwezigheid van HIV specifieke antistoffen bij kind ouder dan 18 maanden.
- Twee maal positieve PCR (HIV-RNA en/of HIV-DNA) op twee verschillende bloedmonsters bij kinderen ouder dan één maand.
- Aanwezigheid van HIV specifieke antistoffen bij kind jonger dan 18 maanden (met uitzondering van navelstrengbloed) in combinatie met een aids definiërende ziekte.
- Een positieve PCR (HIV-RNA en/of HIV-DNA), ook voor de leeftijd van één maand (met uitzondering van navelstrengbloed), in combinatie met een aids definiërende ziekte (15).

HIV negatief bij:

- Afwezigheid van HIV specifieke antistoffen bij kind ouder dan 18 maanden.
- Twee maal negatieve PCR (HIV-RNA en/of HIV-DNA) op twee verschillende bloedmonsters bij kinderen ouder dan één maand, waarvan één afgenomen na de leeftijd van vier maanden.
- En in alle bovenstaande gevallen het ontbreken van klinische of immunologische tekenen van een HIV-infectie en het niet ontvangen hebben van borstvoeding.

Waarschijnlijk HIV negatief bij:

- Eén maal negatieve PCR (HIV-RNA en/of HIV-DNA) bij kinderen ouder dan één maand in combinatie met de afwezigheid van klinische of immunologische tekenen van HIV-infectie, geen borstvoeding.

Onbekend bij:

- Kinderen jonger dan één maand.
- Geen testresultaten bekend.

Resultaten

Gedurende de periode 1995 tot en met 1999 werden bij het NSCK 50 aan HIV geëxposeerde kinderen gemeld, waarbij de HIV infectie van moeder voor of tijdens de zwangerschap was vastgesteld. Voor alle gemelde kinderen werden vragenlijsten ingevuld en geretourneerd. In het retrospectieve onderzoek werden aanvullend 37 aan HIV geëxposeerde kinderen geïncludeerd.

Tabel 1 toont de demografische en klinische kenmerken van de geïncludeerde kinderen (n=87, waaronder 1 gemelli) en hun moeders (n=86).

Een meerderheid van de HIV geïnfecteerde vrouwen (59%) was afkomstig uit een gebied met een gegeneraliseerde HIV epidemie (Afrika ten zuiden van de Sahara of het Caribisch gebied). Acht (9%) kinderen bleken HIV geïnfecteerd te zijn. Het advies om geen borstvoeding te geven lijkt goed opgevolgd te worden: slechts twee kinderen (2%) kregen borstvoeding.

Tabel 2 geeft een overzicht van de acht geïnfecteerde kinderen. Bij drie van de acht HIV geïnfecteerde kinderen was zowel moeder als kind met antiretrovirale therapie behandeld. Géén van de moeders van de geïnfecteerde kinderen was met krachtige antiretrovirale combinatietherapie behandeld. In totaal waren vier kinderen door middel van een sectio caesarea (SC) geboren. Slechts één SC was electief. Een SC geeft alleen een vermindering van transmissie als deze electief, dus voor aanvang van de partus en het breken van de vliezen plaatsvindt. (7, 8) De moeder van het kind

dat door middel van een electieve sectio geboren was, was tijdens de zwangerschap niet behandeld. Waarschijnlijk is hier sprake geweest van een intra-uteriene infectie. Deze hypothese wordt mede gesteund doordat er bij het kind direct na de geboorte een positieve HIV-RNA gevonden werd en doordat er sprake was van een snel progressief klinisch beloop.

Tabel 1: Demografische kenmerken van de aan HIV-1 geëxposeerde kinderen

Kenmerk	Aantal	(%)
Jongens/meisjes	54/33	(62)/(38)
Afkomst moeder (n=86)		
Gebied met een gegeneraliseerde HIV epidemie	51	(59)
Europa Nederland		
Overig	10	(12)
Overig	7	(8)
Onbekend	11	(14)
	7	(8)
Zwangerschapsduur (mediaan, min-max)	38,9 (30,4-42,4) weken	
Geboortegewicht (mediaan, min-max)	3065 (1150-4250) gram	
Partus		
Vaginaal	52	(60%)
Electieve sectio	11	(13%)
Spoedsectio	14	(16%)
Sectio onbekende reden	5	(6%)
Onbekend	5	(6%)
Borstvoeding		
Ja	2	(2)
Nee	82	(94)
Onbekend	3	(3)
HIV-status kind (n=87)		
Positief	8	(9)
Negatief	32	(37)
Waarschijnlijk negatief	42	(48)
Onbekend	5	(6)

Tabel 2: Overzicht van de acht kinderen met HIV-1 infectie

Patiënt	Geboorte-jaar	Therapie moeder	Profylaxe kind	Sectio caesarea (reden)	Borst-voeding	Bijzonder-heden
1	1995	ZDV	ZDV	Nee	Nee	
2	1996	-	-	Nee	Nee	
3	1996	-	ZDV	Ja (foetale nood)	Nee	
4	1997	-	-	Ja (foetale nood)	Nee	
5	1997	ZDV	ZDV /3TC	Nee	Nee	2 dgn post partum gestart met profylaxe
6	1998	ZDV / 3TC	ZDV	Ja (foetale nood)	Nee	
7	1998	-	-	Nee	Ja	
8	1999	-	ZDV /3TC	Ja (electief)	Nee	

Van de 86 moeders werden 67 (77%) gedurende de zwangerschap behandeld met mono-, duo-, triple- of quadrupletherapie (Tabel 3). In alle gevallen kregen kinderen van behandelde moeders ook antiretrovirale therapie. In totaal kregen 81 (93%) kinderen profylaxe gedurende 2 tot 6 weken post partum. De meeste kinderen werden gedurende 4 weken met zidovudine en lamivudine behandeld. In Tabel 3 zijn de verschillende gebruikte behandelingsstrategieën weergegeven.

Tabel 3: Behandelingsstrategieën bij HIV geïnfecteerde moeders en hun kinderen

Therapie			Aantal	(%)
HIV geïnfecteerde moeders				
Geen			18	(21)
Mono			18	(21)
Duo			18	(21)
Triple			29	(33)
Quadruple			2	(2)
Onbekend			1	(1)
Profylaxe kind				
Geen			6	(7)
Mono	zidovudine		27	(31)
	Nevirapine (1 gift)		3	(3)
Duo	zidovudine/lamivudine		47	(54)
	Zidovudine/ lamivudine en 1 gift	nevirapine	2	(2)
Triple			2	(2)

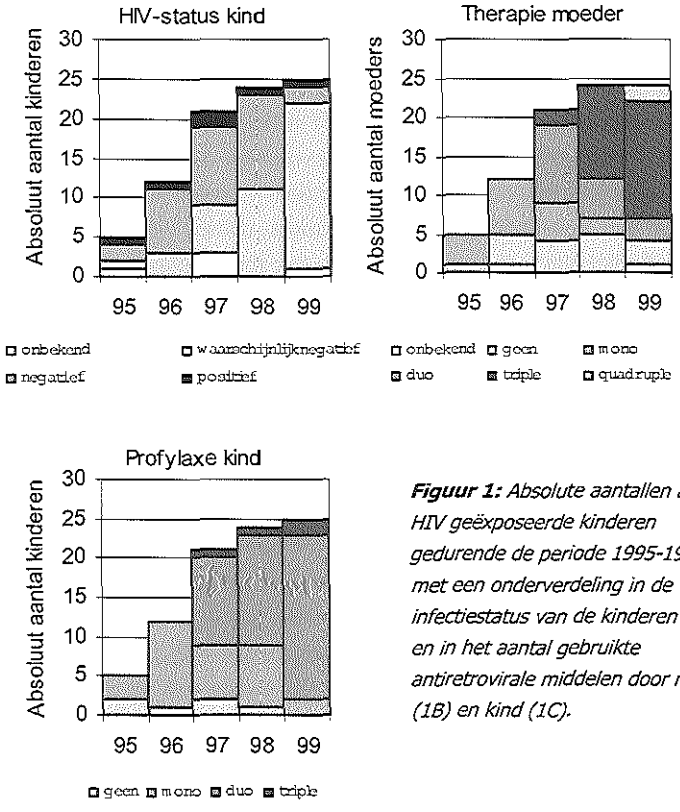
Figuur 1 (A, B en C) laat zien dat gedurende de periode 1995-2000 het absolute aantal bekende aan HIV geëxposeerde kinderen toenam van 5 in 1995 tot 25 in 1999. In dezelfde periode daalde het percentage HIV geïnfecteerde kinderen na bekende expositie van 20% in 1995 tot 4% in 1999 (Figuur 1A). In 1996, 1997 en 1998 waren deze percentages respectievelijk 17, 10 en 8.
Het percentage behandelde moeders bleef gedurende deze periode ongeveer gelijk met een gemiddelde van 70% (range 67%-83%). De samenstelling van de therapie veranderde echter van 80% monotherapie in 1995 tot 0% monotherapie en 70% triple of quadruple therapie in 1999. (Figuur 1B) Ook de samenstelling van de profylactische therapie van de kinderen veranderde van 60% monotherapie in 1995 tot 92% duo of triple therapie in 1999. (Figuur 1C)

Discussie

In de periode 1995 tot en met 1999 is het aantal aan HIV geëxposeerde kinderen waarvan van de moeder ten tijde van de zwangerschap bekend was dat ze HIV geïnfecteerd was in Nederland toegenomen van 5 naar 25 per jaar. Deze stijging is niet gepaard gegaan met een stijging van het aantal kinderen dat na expositie ook HIV geïnfecteerd is geraakt. Het percentage geïnfecteerde kinderen is in deze periode zelfs gedaald van 20% naar 4%. Deze getallen komen overeen met hetgeen te verwachten is op grond van de ontwikkelingen in de kennis over pathogenese en behandeling van HIV-infecties en de daarmee gepaard gaande ontwikkelingen op het gebied van de preventie van verticale transmissie. Het lijkt waarschijnlijk dat de daling van 20% tot 4% in onze populatie voor een groot deel te danken is aan de toename van het gebruik van krachtige

combinatie therapie door de zwangere vrouwen, dat in deze periode steeg van 0% tot 70%. Door de krachtige combinatietherapie wordt een sterkere daling van de virusconcentratie in het plasma bereikt waardoor de kans op virusoverdracht vermindert. Hierbij dient echter aangetekend te worden dat ook bij een ondetecteerbare virusconcentratie in het plasma verticale transmissie beschreven wordt en dat de voorspellende waarde van de virusconcentratie in plasma voor de individuele vrouw slecht is. (12, 13) Helaas ontbreken in onze studie gegevens met betrekking tot de virusconcentratie in het bloed ten tijde van de partus. Sinds 1 januari 2000 zijn deze gegevens opgenomen in de vragenlijst.

Bij de kinderen trad er een verschuiving op van monotherapie naar duotherapie met zidovudine en lamivudine. Dit is een afspiegeling van de invoering van een Nederlands neonatenprotocol waarin deze profylaxe voor de duur van 4 weken post-partum wordt geadviseerd. (10) In de beschreven periode is bij 5% van de kinderen afgeweken van de standaard behandeling met zidovudine en later zidovudine/lamivudine. Afwijken van de standaard profylaxe is een keuze die gemaakt wordt op basis van te verwachten problemen met resistentie. Dit dient vanwege de complexiteit in overleg met een kinderarts uit een kinder-aidsbehandelcentrum (Emma Kinderziekenhuis/Academisch Medisch Centrum Amsterdam, Universitair Medisch Centrum



Figuur 1: *Absolute aantallen aan HIV geëxposeerde kinderen gedurende de periode 1995-1999 met een onderverdeling in de infectiestatus van de kinderen (1A) en in het aantal gebruikte antiretrovirale middelen door moeder (1B) en kind (1C).*

Utrecht/Wilhelmina Kinderziekenhuis, Academisch Ziekenhuis Rotterdam/Sophia Kinderziekenhuis) gedaan te worden.

In totaal werden acht kinderen (9%) met HIV geïnfecteerd ondanks het feit dat het bekend was dat hun moeders tijdens de zwangerschap HIV positief waren. Infectie bij het kind kwam relatief meer voor bij onbehandelde moeders en bij onbehandelde kinderen. Toch werden ook vier kinderen waarbij zowel moeder als kind antiretrovirale middelen had ontvangen met HIV geïnfecteerd. Géén van deze vrouwen had krachtige antiretrovirale combinatietherapie gekregen. Als mogelijke oorzaken voor het falen van de maatregelen kunnen genoemd worden: virologisch falen van het regime met resistentie tegen de gebruikte middelen, therapie-ontrouw, te laat in de zwangerschap gestart met de therapie en vroege intra-uteriene infectie.

De stijging van het aantal bekende HIV geëxposeerde kinderen zou theoretisch kunnen voortkomen uit een stijging van het aantal HIV geïnfecteerde vrouwen afkomstig uit gebieden met een gegeneraliseerde HIV epidemie, een actiever testbeleid onder zwangere vrouwen, een stijging van het aantal vrouwen dat door het succes van krachtige antiretrovirale combinatietherapie een kinderwens heeft of een combinatie van deze factoren. Inzicht in deze problematiek zal de komende jaren verkregen worden doordat de recent aangepaste registratie een vraag bevat over het kader waarin de vrouw getest is.

Ondanks een daling van het percentage HIV geïnfecteerden onder de bekende geëxposeerde kinderen werd er gedurende dezelfde periode in Nederland een stijging waargenomen van het aantal kinderen met een nieuw gediagnosticeerde HIV infectie. Hoewel van het merendeel van deze kinderen één of beide ouders afkomstig was uit een gebied met een gegeneraliseerde HIV epidemie, was het grootste deel van deze kinderen toch in Nederland geboren. (14, 15) Een infectie had derhalve bij een actiever testbeleid onder zwangere vrouwen voorkomen kunnen worden.
In Amsterdam is een universele HIV-screening van zwangere vrouwen kosteneffectief gebleken. (16) Of dit ook voor de rest van Nederland geldt, zal onderzocht moeten worden. Indien een universele HIV screening kosteneffectief blijkt, dan zal bij invoering ook zorg gedragen moeten worden voor het verdwijnen van negatieve gevolgen van de kennis van deze diagnose, bijvoorbeeld met betrekking tot verzekeringsvoorwaarden.

Een actiever testbeleid zal tot gevolg hebben dat meer kinderen van HIV geïnfecteerde moeders, waarvan een groot deel ook zonder interventie niet geïnfecteerd zou raken, zowel intra-uterien als neonataal blootgesteld zal worden aan mogelijk teratogene en toxische antiretrovirale middelen. Een Franse publicatie heeft hier in 1999 opschudding over veroorzaakt (17), maar zekerheid over het bestaan van een associatie tussen de door hen gemelde mitochondriale disfunctie en de antiretrovirale medicatie ontbreekt. Andere studies hebben deze bijwerkingen tot op heden niet kunnen bevestigen. (18, 19) Mocht een associatie met mitochondriale disfunctie bestaan dan zal de afweging die gemaakt moeten worden tussen de nadelen van deze zeer zeldzame, maar ernstige toxiciteit en de voordelen van de grote mate van effectiviteit van perinatale profylaxe bij het voorkomen van een levensbedreigende ziekte uitvallen ten gunste van de voordelen van perinatale profylaxe.

Wij concluderen dat ondanks een stijging van het aantal bekende aan HIV geëxposeerde kinderen er een daling plaatsvindt van het percentage geëxposeerde kinderen dat met HIV geïnfecteerd raakt. In 1999 werd slechts 4% van de geëxposeerde kinderen geïnfecteerd. Hieruit

blijkt dat de in Nederland genomen maatregelen bij HIV positieve zwangere vrouwen en bij hun kinderen effectief zijn.
Het is van groot belang dat de richtlijnen omtrent perinatale profylaxe bekend zijn en nageleefd worden. De registratie zal de komende jaren worden voortgezet waarbij de effectiviteit van de detectie van HIV-positieve zwangeren en de effectiviteit van de perinatale profylaxe bestudeerd zullen worden.

De gegevens waarop dit onderzoek is gebaseerd, werden geleverd door alle kinderartsen in Nederland, van wie wij met name willen noemen: mw. dr. S.P.M. Geelen, Universitair Medisch Centrum Utrecht/Wilhelmina Kinderziekenhuis, Utrecht en dr. N.G. Hartwig, Academisch Ziekenhuis Rotterdam/Sophia Kinderziekenhuis, Rotterdam.

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Epidemiology of HIV-1 infected children in The Netherlands

A.M.C. van Rossum, R.A. Hirasing, R. de Groot

Ned Tijdschr Geneeskd (in press)

Chapter 4

Abstract

Objective: Prospective registration of the number of children diagnosed with aids and/or HIV infection. *Design:* Prospective registration from January 1 1998 to December 31 2000.
Methods: Dutch paediatricians monthly reported HIV positive children to the Dutch Paediatric Surveillance Unit. All reports were followed up with standard questionnaires. *Results:* During the period 1998-2000 42 children were diagnosed with HIV-1 infection. This number was almost equal to the number of HIV-1 infected children diagnosed during the period 1995-1997 (n=43). In 86% of the children one or both parents originated from a country with a generalised HIV epidemic. Of only 2 children (5%) both parents originated from The Netherlands. Most children (81%) were infected by mother to child transmission. Of these children 48% (n=20) was born in The Netherlands. Only 33% of the HIV-1 infected children lived in one of the four major cities of The Netherlands. *Conclusions:* The increase in the number of newly detected HIV-1 infected children during the period 1995-1997 reached a plateau during the period 1998-2000. This may be due to a more active approach in the testing on HIV in pregnant women. Forty-eight % (n=20) of the children infected by mother to child transmission were born in The Netherlands. The risk on transmission of HIV could have been significantly reduced when the mother had been tested on an HIV infection and treated according to the current guidelines.

Samenvatting

Doel: Registratie van het aantal met een HIV infectie gediagnosticeerde kinderen in Nederland.
Opzet: Prospectieve registratie in de periode 1 januari 1998 tot 31 december 2000. *Methode:* Nederlandse kinderartsen meldden maandelijks via het Nederlands Signaleringscentrum Kindergeneeskunde ieder met een HIV infectie gediagnosticeerd kind. De meldingen werden gevolgd door toezending van vragenlijsten. *Resultaten:* In de periode van 1998 tot en met 2000 werd bij 42 kinderen een HIV-1 infectie vastgesteld. In de periode 1995-1997 was dit aantal nagenoeg gelijk (n=43). Bij 86% van de kinderen was één of beide ouders afkomstig uit een gebied met een gegeneraliseerde HIV epidemie. Van slechts 2 kinderen (5%) waren de beide ouders van Nederlandse afkomst. Het merendeel (81%) van de kinderen werd via verticale transmissie door hun moeder geïnfecteerd. Van deze kinderen was 48% (n=20) in Nederland geboren. Slechts 40% van de in Nederland geboren en door verticale transmissie geïnfecteerde kinderen woont in één van de vier grote steden. De overige 60% woont verspreid over nagenoeg alle provincies van Nederland.
Conclusies: De stijging van het aantal kinderen dat in de periode 1995-1997 met een HIV infectie werd gediagnosticeerd, lijkt af te vlakken in de periode van 1998 tot en met 2000. Dit is mogelijk een gevolg van een actiever testbeleid onder zwangere vrouwen uit risicogroepen. Van de kinderen die geïnfecteerd waren door verticale transmissie was 48% (n=20) in Nederland geboren. De kans op een HIV infectie zou bij deze kinderen aanzienlijk lager geweest zijn indien hun moeder tijdens de zwangerschap getest zou zijn op de aanwezigheid van een HIV infectie gevolgd door het instellen van een beleid gericht op het voorkomen van verticale transmissie.

Inleiding

Na een periode waarin weinig kinderen met HIV werden gediagnosticeerd, werd vanaf het begin van de jaren '90 een toename vastgesteld van het aantal meldingen bij kinderen in Nederland. (1) Twee oorzaken waren verantwoordelijk voor deze stijging. De eerste hiervan was de toename van het aantal door heteroseksueel contact met HIV geïnfecteerde zwangere vrouwen. (2) Indien een zwangere vrouw met HIV geïnfecteerd is, is de kans op overdracht van HIV van moeder op kind tijdens de zwangerschap en perinataal 15-30% indien geen interventie plaats vindt met antiretrovirale middelen. (3-6) Daarnaast hebben zich gedurende de afgelopen jaren mensen in Nederland gevestigd die afkomstig zijn uit gebieden met een gegeneraliseerde HIV-epidemie.(Afrika ten zuiden van de Sahara en het Caribische gebied). In tegenstelling tot de situatie in de Westerse wereld wordt HIV in deze gebieden voornamelijk heteroseksueel verspreid. Geschat wordt dat in Afrika 10,9 miljoen mannen en 13,3 miljoen vrouwen met HIV geïnfecteerd zijn. (7)

Om de HIV epidemie onder kinderen in Nederland te monitoren, is sinds 1995 via het Nederlands Signalerings-Centrum Kindergeneeskunde (NSCK), aanvankelijk tezamen met de afdeling kindergeneeskunde van het Universitair Medisch Centrum Utrecht/Wilhelmina Kinderziekenhuis en vanaf 1998 in samenwerking met het Academisch Ziekenhuis Rotterdam/Sophia Kinderziekenhuis, het aantal HIV positieve kinderen prospectief geregistreerd. De resultaten tot en met 1997 zijn eerder in dit tijdschrift gepubliceerd. (1) In dit artikel worden de resultaten in de periode van 1998 tot en met 2000 beschreven en vergeleken met de gegevens uit de voorgaande periode.

Patiënten en methoden

Met ingang van 1 januari 1995 melden praktiserende kinderartsen maandelijks elke nieuwe patiënt (0-18 jaar) met tenminste één van de volgende inclusiecriteria bij het NSCK: bewezen aids, positieve HIV serologie, positieve viruskweek, positieve polymerasekettingreactie (PCR) op HIV-RNA, positieve p24 antigeenbepaling of een pasgeborene van een HIV positieve moeder met een nog onbekende HIV serostatus. Na melding van een HIV geïnfecteerd kind of een aan HIV geëxposeerd kind wordt de betreffende kinderarts verzocht een vragenlijst in te vullen met vragen over klinische en sociale kenmerken van het gemelde kind. Alle gegevens werden anoniem verwerkt.

In dit artikel worden de resultaten beschreven van de registratie van met HIV gediagnosticeerde kinderen in de periode 1 januari 1998 tot 31 december 2000. De resultaten van de aan HIV geëxposeerde kinderen (zowel HIV negatieve als HIV geïnfecteerde kinderen) worden in een afzonderlijk artikel gepubliceerd.

De HIV-status van de met HIV infectie gediagnosticeerde kinderen werd als volgt gedefinieerd:
HIV geïnfecteerd bij:

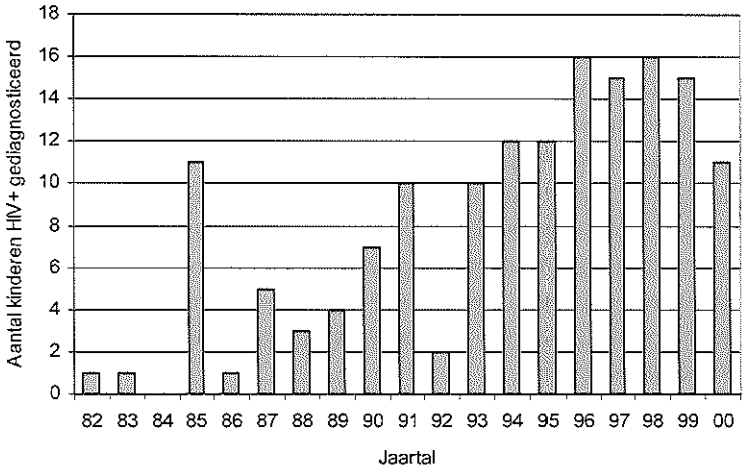
- Aanwezigheid van HIV specifieke antistoffen bij kind ouder dan 18 maanden.
- Twee maal positieve PCR (HIV-RNA en/of HIV-DNA) op twee verschillende bloedmonsters bij kinderen ouder dan één maand.
- Aanwezigheid van HIV specifieke antistoffen bij kind jonger dan 18 maanden (met uitzondering van navelstrengbloed) in combinatie met een aids definiërende ziekte.
- Een positieve PCR (HIV-RNA en/of HIV-DNA), ook voor de leeftijd van één maand (met uitzondering van navelstrengbloed), in combinatie met een aids definiërende ziekte (8).

De resultaten van de registratie in de periode 1998 tot en met 2000 werden vergeleken met de resultaten van de registratie in de periode 1982 tot en met 1997.

Resultaten

Gedurende de periode 1998-2000 werden 42 kinderen bij het NSCK gemeld. Van alle kinderen werden vragenlijsten door de meldend kinderarts ingevuld en geretourneerd. Sinds begin jaren negentig wordt de zorg voor HIV geïnfecteerde kinderen voornamelijk geconcentreerd in drie kinderaidsbehandelcentra (Academisch Medisch Centrum/Emma Kinderziekenhuis, Amsterdam, Universitair Medisch Centrum/Wilhelmina Kinderziekenhuis, Utrecht, en Academisch ziekenhuis Rotterdam/Sophia Kinderziekenhuis, Rotterdam). Hierdoor is met grote mate van zekerheid vast te stellen dat het aantal meldingen ook daadwerkelijk overeenkomt met het aantal met HIV gediagnosticeerde kinderen.

Figuur 1 toont het aantal kinderen dat per jaar met HIV gediagnosticeerd werd. In de periode 1995-1997 werd een toename van het aantal met HIV gediagnosticeerde kinderen vastgesteld ten opzichte van de jaren daarvoor. Deze toename lijkt tot stilstaan gekomen te zijn in de periode 1998-2000. In de periode 1995-1997 werd bij 43 kinderen een HIV infectie vastgesteld tegen 42 in de periode 1998-2000. In alle gevallen ging het om een infectie met HIV type 1. Infectie met HIV type 2 werd in de periode 1982-2000 in Nederland slechts één maal bij een kind gediagnosticeerd.



Figuur 1: Het aantal met HIV gediagnosticeerde kinderen in Nederland gedurende de periode 1982-2000

Tabel 1 geeft een aantal geselecteerde kenmerken aan van de met HIV gediagnosticeerde kinderen. Verticale transmissie was evenals in de voorgaande periode de meest voorkomende wijze van infectie overdracht. Van de kinderen die in de periode 1998-2000 gediagnosticeerd werden, werd

81% geïnfecteerd door verticale transmissie. In de periode 1995-1997 werd 58% van de via verticale tranmissie met HIV geïnfecteerde kinderen in Nederland geboren. In de periode 1998-2000 was dit percentage 48%. Zeventig procent van de moeders van kinderen die in Nederland geboren waren en door verticale transmissie geïnfecteerd waren, was afkomstig uit een gebied met een gegeneraliseerde HIV epidemie, 15% uit Nederland en 15% uit andere landen buiten Nederland (Marokko, Phillipijnen).

Tabel 1: Kenmerken van de 159 met HIV gediagnosticeerde kinderen in Nederland

	HIV+ t/m 1994 n=74		HIV+ 1995-1997 n=43		HIV+ 1998-2000 n=42	
Jongens/meisjes	37/37		29/14		21/21	
Overleden op moment van melding	26	(36%)	7	(16%)	0	(0%)
In Nederland geboren	50	(69%)	27	(63%)	20	(48%)
Afkomst						
Beide ouders Nederlands	34	(46%)	5	(12%)	2	(5%)
Eén of beide ouders uit een gebied met een gegeneraliseerde HIV epidemie	29	(39%)	33	(77%)	35	(83%)
Eén of beide ouders uit een ander Europees land dan NL	6	(8%)	2	(5%)	2	(5%)
Anders	4	(7%)	3	(7%)	3	(7%)
Onbekend						
Wijze van infectie						
Verticale transmissie	43	(58%)	31	(72%)	34	(81%)
Hemofilie	12	(16%)				
Bloed (producten) transfusie	14	(19%)	3	(7%)	1	(2%)
Prikaccident						
Chirurgische ingreep	1	(1%)	1	(2%)		
Seksueel contact/misbruik	1	(1%)			3	(7%)
Onbekend	3	(4%)	8	(19%)	4	(10%)
Reden tot testen kind						
Kind vertoonde symptomen van HIV Infectie	20	(28%)	27	(63%)	27	(64%)
Moeder of beide ouders zijn HIV Geïnfecteerd	28	(39%)	11	(26%)	8	(19%)
Een ander familielid is HIV Geïnfecteerd			1	(2%)	2	(5%)
Getest na hemofilie behandeling	7	(10%)				
Tropenscreening			1	(2%)	5	(12%)
Onbekend	17	(23%)	3	(7%)		

Bij 64% van de kinderen werd een HIV-test verricht, omdat het kind symptomen van een HIV infectie vertoonde en bij 19% omdat moeder bekend was met een HIV infectie. Van de acht kinderen waarvan het HIV seropositief zijn van de moeder de reden tot testen was, was van drie

kinderen al voor de geboorte bekend dat de moeder met HIV geïnfecteerd was. Slechts één van deze moeders werd tijdens de zwangerschap behandeld (met zidovudine en lamivudine). Hierop wordt nader ingegaan in ons artikel over de verticale transmissie bij aan HIV geëxposeerde kinderen (Van Rossum et al. accepted Ned. Tijdschr. Geneeskd.).

In tabel 2 is de leeftijd weergegeven waarop de diagnose werd gesteld bij in Nederland geboren en door verticale transmissie geïnfecteerde kinderen. Bij 75% van kinderen werd de diagnose vóór de leeftijd één jaar gesteld. Dit komt overeen met hetgeen te verwachten is op grond van het bekende hoge percentage HIV geïnfecteerde kinderen dat in het eerste levensjaar symptomen van een HIV infectie vertoont. (9)

Tabel 2: Leeftijd van door verticale transmissie geïnfecteerde en in Nederland geboren kinderen op het moment van stellen van de diagnose

Leeftijd	Diagnose in periode		
	1982-1994 n=26	1995-1997 n=25	1998-2000 n=20
< 1 jaar	19 (73%)	15 (60%)	15 (75%)
1-2 jaar	2 (8%)	6 (24%)	2 (10%)
3-5 jaar	2 (8%)	4 (16%)	2 (10%)
≥ 6 jaar	2 (8%)	0	1 (5%)
Onbekend	1 (4%)	0	

Op basis van postcodes (alleen de cijfers van de postcode) werd geanalyseerd uit welke stad of provincie (indien de woonplaats niet één van de vier grote steden
- Amsterdam, Den Haag, Rotterdam en Utrecht - was) de met HIV gediagnosticeerde kinderen afkomstig waren. Opvallend is dat slechts een derde van de kinderen afkomstig is uit één van de vier grote steden. De overige kinderen zijn afkomstig uit nagenoeg alle provincies in Nederland. Van de in Nederland geboren en door verticale transmissie geïnfecteerde kinderen woonde 40% in één van de vier grote steden en 60% verspreid over zeven provincies.

Discussie

De stijging van het aantal nieuw gediagnosticeerde kinderen met een HIV infectie die in de periode tot en met 1997 werd vastgesteld, is gestopt in de periode 1998 tot en met 2000. In beide periodes werden nagenoeg evenveel kinderen met een HIV infectie gediagnosticeerd (43 in de periode 1995-1997 en 42 in de periode 1998-2000).

Bij 86% van de kinderen was één of beide ouders afkomstig uit een gebied met een gegeneraliseerde HIV epidemie. Van slechts 2 kinderen (5%) waren de beide ouders van Nederlands afkomst. Dit suggereert dat de stijging van het aantal HIV infecties bij kinderen de afgelopen jaren niet zozeer een afspiegeling is van een toename van het aantal HIV geïnfecteerde vrouwen van Nederlandse afkomst, maar met name veroorzaakt wordt doordat zich mensen in Nederland gevestigd hebben afkomstig uit gebieden met een gegeneraliseerde HIV-epidemie.

Evenals in de periode 1995-1997 werd het merendeel (81%) van de kinderen via verticale transmissie door hun moeder geïnfecteerd. Van deze kinderen was 48% (n=20) in Nederland geboren. De kans op een HIV infectie had bij deze kinderen aanzienlijk gereduceerd kunnen worden indien hun moeder tijdens de zwangerschap getest zou zijn op de aanwezigheid van een HIV infectie. Indien de HIV infectie van moeder tijdens de zwangerschap bekend is, bestaat de mogelijkheid tot medicamenteuze interventie bij zowel moeder als kind en eventueel een sectio caesarea in plaats van een vaginale partus als er ten tijde van de partus een meetbare hoeveelheid virus in het bloed aanwezig is. Het transmissiepercentage kan hiermee gereduceerd worden van 15-30% tot 1-2 %. (10-13)

Voor volwassenen geldt dat met name de vier grote steden (Amsterdam, Den Haag, Rotterdam en Utrecht) een relatief hoge prevalentie van HIV hebben. In 1997 werd Amsterdam voor volwassenen betiteld als epicentrum van de HIV epidemie in Nederland. (14) De woonplaatsen van de HIV geïnfecteerde kinderen liggen echter verspreid over het gehele land. Slechts 33% van de kinderen woont in één van de vier grote steden. De overige 67% woont verspreid over nagenoeg alle provincies van Nederland. De grotere spreiding van HIV geïnfecteerde kinderen is waarschijnlijk mede veroorzaakt door de verspreiding van asielzoekers over alle Nederlandse provincies. Deze spreiding brengt problemen met zich mee indien de kinderen in een stadium van de HIV infectie komen waarin behandeling met antiretrovirale middelen noodzakelijk is. Deze behandeling is zeer complex en vereist frequente bezoeken aan één van de drie kinderaidsbehandelcentra. Dit legt een grote druk op gezinnen in een moeilijke sociale en financiële situatie. Het centreren van HIV geïnfecteerde kinderen in de omgeving van één van de drie kinderaidsbehandelcentra verdient derhalve aanbeveling. Daarnaast is de ontwikkeling van zorgprotocollen om de samenwerking tussen eerste, tweede en derde lijn te optimaliseren noodzakelijk om kinderen die verder van de centra af wonen toch optimale zorg te bieden.

Aangezien het aantal patiënten met HIV/aids in gebieden met een gegeneraliseerde HIV epidemie nog steeds toeneemt, zal het percentage HIV geïnfecteerde vrouwen dat zich vanuit deze gebieden in Nederland vestigt naar verwachting verder stijgen. Dit kan resulteren in een stijging van het aantal HIV geïnfecteerde kinderen dat door verticale transmissie geïnfecteerd raakt. Het feit dat deze stijging tot op heden is uitgebleven, is mogelijk het gevolg van het actiever aanbieden van een HIV test tijdens de zwangerschap gevolgd door interventie bij een positief testresultaat. Ondanks een gelijk aantal met een HIV infectie gediagnosticeerde kinderen in de periodes 1995-1997 en 1998-2000 is een daling waar te nemen in het aantal kinderen dat door verticale transmissie geïnfecteerd is en in Nederland geboren is. In de periode 1995-1997 werden 25 kinderen in Nederland geboren en door verticale transmissie geïnfecteerd. In de periode 1998-2000 was dit gedaald tot 20 kinderen.

Wij concluderen dat de stijging van het aantal nieuw gediagnosticeerde kinderen met een HIV infectie die in de periode tot en met 1997 werd vastgesteld lijkt af te vlakken in de periode van 1998 tot en met 2000. Mogelijk is dit een gevolg van een actiever testbeleid onder zwangere vrouwen uit risicogroepen. Van de kinderen geïnfecteerd door verticale transmissie was 48% (n=20) in Nederland geboren. De kans op een HIV infectie had bij deze kinderen aanzienlijk gereduceerd kunnen worden indien hun moeder tijdens de zwangerschap getest zou zijn op de aanwezigheid van een HIV infectie, gevolgd door een antiretrovirale behandeling van moeder en kind volgens de daartoe ontwikkelde protocollen. (15)

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Part II

Pharmacology of antiretroviral agents in children with HIV-1 infection

Pharmacokinetics of the protease inhibitor indinavir in children with HIV-1 infection

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Chapter 5

Abstract

Objective: To evaluate the pharmacokinetics of indinavir in HIV-infected children as part of a prospective, open, uncontrolled, multicentre study in the Netherlands. *Study design:* HIV-1-infected children were followed for 6 months treatment with zidovudine 120mg/m2 q8h, lamivudine 4 mg/kg q12h and indinavir 33mg/kg metabolic weight (MW) q8h. Four weeks after start of treatment, the steady state pharmacokinetics of indinavir were determined by HPLC. If patients had an indinavir AUC below 10 or above 30 mg/L.h a dose increment or a dose reduction was made and pharmacokinetic measurements were repeated four weeks later. *Results:* 19 Patients started with the 33mg/kg MW q8h dose. The median (+ range) AUC was 10.5 (2.8-51.0) mg/L.h. The median AUC in 17 children treated with 50 mg/kg MW q8h was 20.6 (4.1-38.7) mg/L.h. Finally, 5 patients had a dose increment to 67 mg/kg MW q8h, resulting in a median AUC of 36.6 (27.2-80.0) mg/L.h. After 6 months of treatment, there were 11 children with an AUC below 20 mg/L.h of whom 5 (45%) had a detectable viral load, while this was the case in none of the 11 children with an AUC higher than 20 mg/L.h. *Conclusion:* The optimal dose of indinavir in children to obtain drug exposure similar to that observed in adult patients is 50 mg/kg MW q8h, which approximates 600mg/m^2 q8h. It would even be better to adjust the indinavir dose based on an AUC greater than 20 mg/L.h.

Introduction

The advent of triple drug therapy, which includes two nucleoside reverse transcriptase inhibitors and one protease inhibitor or two nucleoside reverse transcriptase inhibitors and one non-nucleoside reverse transcriptase inhibitor, has markedly changed therapeutic options for HIV-infected individuals. So far, the large majority of clinical trials has been conducted in HIV-1 infected adults, whereas the use of triple drug combination therapy in children has only recently been reported (9,12-14, 17,19).

Differences in pharmacokinetics between children and adults should warn investigators that successful medical treatment in adults does not necessarily imply that this therapy will also be of benefit in children. Therefore, the use of new agents, such as the HIV-1 protease inhibitors, should be guided by detailed pharmacokinetic and pharmacodynamic evaluations when given for the first time to children. The goal of this approach is that efficacy and toxicity data should be similar to those reported in adults, or even better, if possible. With regard to HIV infection, suboptimal therapy should be detected as soon as possible, and interventions (e.g., dose modifications) should be applied immediately to prevent the emergence of drug resistance.

We here report the pharmacokinetics of the HIV-protease inhibitor indinavir in children participating in a prospective, open, uncontrolled clinical trial. A detailed analysis of the clinical, immunological and virological effects is presented elsewhere (16).

Materials and Methods

Patients

HIV-1 infected children between the age of 3 months and 18 years with a viral load greater than 5,000 copies/mL (Roche Amplicor®) and/or a CD4 cell count below an age-specific threshold were included between April 1997 and July 1998. The study protocol was approved by the Medical Ethics

Committees of all participating hospitals and written informed consent was obtained from all parents or legal guardians.

Pretreatment with zidovudine, didanosine and/or zalcitabine was allowed. Patients were followed for 6 months treatment of zidovudine 120mg/m2 q8h, lamivudine 4 mg/kg q12h and indinavir 33mg/kg metabolic weight q8h. The indinavir dose was based on the assumption that the use of metabolic weight (MW = body weight$^{0.75}$) would better reflect the higher body clearance of drugs in children as compared to adults (10). An average adult weighing 70kg has a MW of 24 kg (= $70^{0.75}$ kg); the usual adult dose of indinavir is 800mg q8h, which is equal to 33 mg/kg MW (800 mg / 24 kg MW) q8h.

Indinavir was administered as 200mg or 400mg capsules (Crixivan®) or as 150mg or 300mg capsules (prepared by the hospital pharmacy). If patients were not able to swallow the capsules, the indinavir capsules were opened and mixed with 5-10mL of water. Patients and caregivers were instructed to take the indinavir capsules on an empty stomach, or with a low-energy meal.

Pharmacokinetics

Four weeks after start of treatment, patients were admitted to the day-care unit of each hospital to determine the steady state pharmacokinetics of indinavir. Patients took indinavir on an empty stomach and blood samples were collected just before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, and 8 hours post ingestion. Plasma was separated by centrifugation and samples were stored at –20 °C until analysis. Indinavir concentrations were determined in plasma by HPLC, as previously reported (2). In brief, 100 µL of plasma and 10 µL of an internal standard (125 µg/mL of methylindinavir dissolved in acetonitrile) were added to 400 µL of acetonitrile. Each cup was vortexed for 1 minute and subsequently centrifuged for 5 minutes at 10,500 g. The supernatant was evaporated to dryness. The residue was dissolved in the eluent which consisted of acetonitrile-water (40:60, v/v). The water phase contained 50 mM phosphate buffer pH 6 and 4 g/L tetramethylammoniumchloride. The analytical column was an Inertsil™ ODS-2 C_{18} column. Ultraviolet absorption was monitored at 210 nm.With this assay indinavir plasma concentrations can be measured between 0.05 - 12.5 mg/L. If the plasma concentration was higher than 12.5 mg/L, the sample was 1:1 diluted with drug-free plasma and re-analyzed. This dilution protocol was validated. At an indinavir concentration of approximately 1 mg/L the accuracy of the assay is 97.7% with an inter- and intra-assay variation of 3.1% and 4.8%, respectively.

Pharmacokinetic parameters were calculated in Microsoft Excel® 97 by non-compartmental methods (6). The highest observed plasma concentration was defined as C_{max}, with the corresponding sampling time as T_{max}. The terminal, log-linear period (Log C versus T) was defined by the last data points (n > 2) by visual inspection. The absolute value of the slope (β/ln10) was calculated by least-square analysis. The elimination half-life ($t_{½}$) was calculated using the equation $t_{½}=\ln 2/\beta$. The Area under the C versus T curve (AUC) was calculated by using the trapezoidal rule for t_0 tot t_8. The target $AUC_{0\text{-}8h}$ of indinavir was based on adult values of 20 ± 10 mg/L.h (1) & (unpublished observations). If patients had an indinavir AUC outside this range a dose modification of 50-200% was made and pharmacokinetic measurements were repeated four weeks later.

Pharmacokinetics parameters in the children were compared to a population of 15 HIV-1 infected adults who are treated at the University Hospital Nijmegen. These patients were using

indinavir 800mg q8h as part of a triple drug regimen for at least 4 weeks. The patients were randomly selected from the outpatient population to assess the population pharmacokinetics of indinavir in adults. Patients took 800mg of indinavir on an empty stomach and blood samples were collected just before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, and 8 hours post ingestion.

Pharmacokinetic/pharmacodynamic relationships

The relation between the plasma concentrations of indinavir and its therapeutic effect was investigated in patients who completed the 6 months period of the study. For those patients the last recorded AUC was related to the viral load response at 6 months. The group of patients was divided in two, based on an indinavir AUC higher or lower than the median value observed in adults (approximately 20 mg/L.h), and for each group the number of patients was counted that had an undetectable viral load (< 500 copies/mL).

Statistics

All statistical tests were performed using SPSS for Windows version 8.0 (SPSS Inc. Chicago, Il, USA). Mann-Whitney U test and Pearson's Chi-Square test were used for comparison of pharmacokinetic parameters between subgroups.

Results

Inclusion

A total number of 27 patients was included in the prospective clinical trial. An 8-hour pharmacokinetic curve was recorded at least once in 25 (12 M and 13 F) of them. Two of the children stopped taking indinavir before a pharmacokinetic curve could be recorded at week 4. The median age of the 25 children was 6.0 years (range: 3 months to 16 years).

Pharmacokinetics of indinavir

19 Patients started with the 33mg/kg MW q8h dose. The median (+ range) $AUC_{0\text{-}8h}$, was 10.5 (2.8-51.0) mg/L.h. Other pharmacokinetic parameters are listed in table 1. For comparison, average values from a population of 15 HIV-infected adults using 800mg of indinavir q8h are listed in the table too. Because the AUC was below 10 mg/L.h in 11 of the children, the indinavir dose was increased to 50mg/kg MW q8h in these patients (150% dose). In addition, 6 other children started treatment with this higher dose. The median (+ range) $AUC_{0\text{-}8h}$ in these 17 children was 20.6 (4.1-38.7) mg/L.h (see also table 1). Finally, 5 patients had a dose increment to 67 mg/kg MW q8h (200% dose), resulting in a median (+ range) $AUC_{0\text{-}8h}$ of 36.6 (27.2-80.0) mg/L.h. Mean plasma concentrations of indinavir in the three dosing groups in children as well as the reference data for adults are depicted in figure 1.

The weight-corrected apparent oral clearance of indinavir in children using the 33mg/kg MW qh8 dose was used to investigate factors that were related to the huge variation in the AUC values (coefficient of variation 85%). The median apparent oral clearance (+ range) of indinavir in the 19 children who started to use indinavir in the 33 mg/kg MW q8h dose level was 1.1 (0.3-4.6) L/(h.kg). There was no statistically significant difference between boys (n = 8) and girls (n = 11): 1.6 (0.3-4.6) vs. 1.1 (0.6-4.1) L/(h.kg)(p=0.96, Mann Whitney U Test). Although the variation in

clearance values was much larger in younger children than in older children, children below the median age of 6 years (n = 9) had a significantly higher apparent oral clearance of indinavir than children aged 6 years and older (n = 10): 2.5 vs. 1.0 L/(h.kg)(p=0.03, Mann Whitney U Test). The average adult value is 0.6 L/(h.kg). There were no medications that were concomitantly used with indinavir that are known or suspected to have an influence on indinavir clearance.

Table 1. Pharmacokinetic parameters of indinavir

Parameter	Children			Adults
Indinavir dose (q8h)	33 mg/kg MW	50 mg/kg MW	67 mg/kg MW	800 mg
n =	19	17	5	15
$AUC_{0\text{-}8h}$ (mg/L.h)	10.5 (2.8-51.0)	20.6 (4.1-38.7)	36.6 (27.2-80.0)	19.1 (8.5-33.3)
CL/F (L/(h.kg))	1.1 (0.3-4.6)	0.9 (0.5-4.7)	0.6 (0.4-1.2)	0.6 (0.4-1.4)
Vd/F (L/kg)	2.3 (0.5-11.5)	1.7 (0.5-6.8)	1.1 (0.3-1.7)	1.3 (0.6-3.2)
Cmax (mg/L)	6.4 (2.1-19.5)	9.7 (2.3-17.0)	17.1 (15.0-29.0)	8.7 (3.6-15.8)
Tmax (h)	1.0 (0.5-2.0)	1.0 (0.5-2.5)	0.8 (0.4-1.6)	1.0 (0.5-2.0)
Cmin (mg/L)	0.04 (<0.02-0.26)	0.07 (0.02-0.21)	0.17 (0.05-0.38)	0.13 (0.03-0.29)

Data are median values + ranges between brackets

Pharmacokinetic-pharmacodynamic relationships

The relation between the plasma concentration of indinavir and the antiviral effect of the treatment regimen was investigated in the 22 children who completed 6 months of treatment and in whom the pharmacokinetic parameters were available for the dose that they were using at that timepoint. There was no statistically significant difference between the AUC, the C_{max}, or the C_{min} values in the patients who responded to therapy (defined as a viral load below 500 copies/mL) and those who did not respond to therapy (defined as a viral load above 500 copies/mL). However, there were 11 children with an AUC below 20 mg/L.h in whom 5 (45%) had a detectable viral load after 6 months of treatment, while this was the case in none of the 11 children with an AUC higher than 20 mg/L.h (p=0.01, Pearson Chi-Square test, see figure 2).

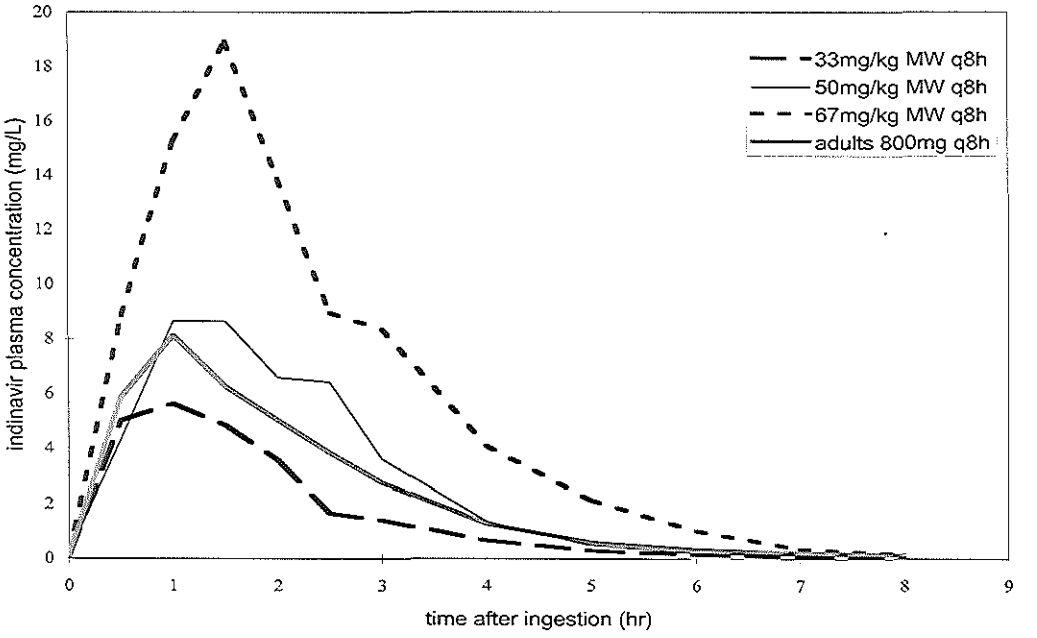

Figure 1: ***Mean plasma concentrations of indinavir vs. time of the 3 dose levels and the comparison adult data***

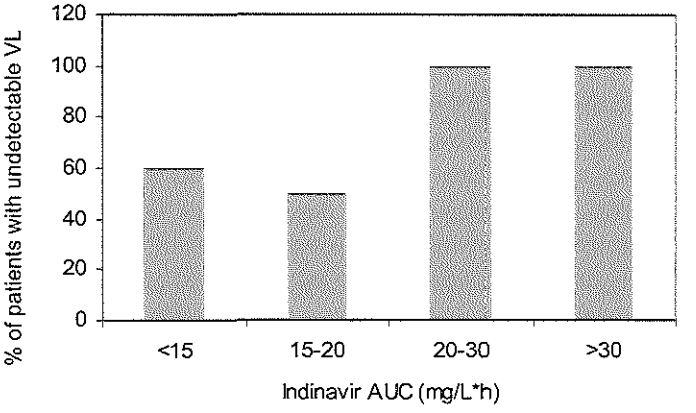

Figure 2: *The relation between the AUC of indinavir and the virological response after 6 months of treatment. The number of patients in the AUC groups are: 5 (< 15 mg/L.h); 6 (15-20 mg/L.h); 7 (20-30 mg/L.h); 4 (> 30 mg/L.h).*

Discussion

The optimal dose of indinavir for HIV-infected children has not yet been established. The first phase I/II study was performed using 3 different formulations: two kinds of suspensions of indinavir base and dry-filled capsules of indinavir. Because of the poor absorption of indinavir from the suspensions, further research was conducted only with the capsules containing indinavir sulphate, now marketed as Crixivan® capsules (9,14,15,19). Most children have been treated with an indinavir dose of 500mg/m^2 q8h although this dose had to be reduced to 350mg/m^2 q8h in the phase I/II study because of a high incidence of nephrolithiasis in children using the higher dose (14).

We have evaluated three dose levels of indinavir in this study. We started with a dose of 33 mg/kg MW q8h, but this resulted in low AUC values in a substantial proportion of the children. A dose increment to 50 mg/kg MW q8h resulted in AUC values that were comparable to values observed in adults. Five children received the highest indinavir dose of 67 mg/kg MW qh8, but this led to large increases in indinavir plasma concentrations and serious toxicity (nausea, vomiting) in these children. Therefore, the optimal dose appeared to be 50 mg/kg MW q8h. After 6 months of treatment 16 out of the 25 patients who are still on indinavir treatment are now using this dose. 70 Percent of these children have reached the goal of an undetectable viral load (< 500 copies/mL).

We have chosen to use the MW of a child to calculate the indinavir dose. The use of MW instead of normal body weight is based on the assumption that metabolic clearance in children is higher than in adults, even when corrected for body weight. Although we had anticipated this higher oral clearance of indinavir in children, we made the wrong assumption that we should use an adult of 70kg to calculate the indinavir dose of 33 mg/kg MW (=800mg/$70^{0.75}$ kg. For most medications, children with an age of 10-12 years already receive the adult dose. In retrospect, it would have been better to use an estimated body weight of 45 kg of an 12 year old child to calculate the indinavir dose: MW is then 17.3 kg, 800mg divided by 17.3 kg MW results in an indinavir dose of 46 mg/kg MW q8h. This is close to the 50 mg/kg MW that we have found in this study to be the optimal dose of indinavir. This dose results in a median AUC value of 20.6 mg/L.h which is just above the median adult value of 19 mg/L.h. An indinavir dose of 500mg/m^2 q8h is now under investigation in a phase III clinical trial (15). Both methods used to calculate pediatric doses, i.e. metabolic weight and body surface area, are based on similar physiological mechanisms. Because pediatricians are more familiar with dosing based on body surface are, this may be preferred over dosing on metabolic weight. The 50mg/kg MW q8h that we found to be the optimal dose approximates a dose of 600mg/m^2 q8h.

Not only a higher metabolic clearance of indinavir in children may have resulted in the differences in clearance values between children and adults. Because indinavir is administered orally, impaired absorption of the drug may result in higher apparent oral clearances of indinavir. The reasons for possible malabsorption may be variable, but one of the mechanisms may be reduced solubility of indinavir at relatively high gastric pH values (11). Because it is known that children younger than 3 years have reduced gastric acid secretion (4), this may result in lower indinavir exposure. However, peak concentrations of indinavir were achieved rapidly and were often higher than what is seen in adults. Furthermore, dose increments would not lead to increased plasma concentrations of indinavir if drug solubility is problematic. In contrast, more than proportional

increases in C_{max} and AUC were seen (table 1). This makes impaired absorption due to increased gastric pH unlikely.

Yet another possible explanation of lower plasma levels of indinavir may be an increased volume of distribution. Young children have more total body water (80-90% of body weight) than adults (55-60% of body weight)(4), so water-soluble drugs, such as indinavir, will have a larger apparent volume of distribution in children than in adults (table 1).

In adults there is a number of observations showing that the height of the plasma concentration of indinavir is related to the antiviral response (3,17,18). Therefore, instead of using a fixed dose regimen for each child, it seems logical to monitor the plasma levels of indinavir shortly after the start of an indinavir-containing regimen and adjust the indinavir dose if deemed necessary. For adults a trough level of 0.1 mg/L has been proposed as the minimum effective concentration, which is equal to 75% of an average population value (3,8). These figures can be extrapolated to an AUC value of approximately 15 mg/L.h (75% of 20), but our data show that for children this will not be enough. The AUC threshold in children appears to be 20 mg/L.h, because none of the children with an AUC higher than 20 mg/L.h had a detectable viral load after 6 months of treatment (figure 2). Not all the children with an AUC below this threshold of 20 mg/L.h can be considered as non-responders, but it is clear that the risk of virological failure is much greater with these lower AUC values. The fact that a higher AUC of indinavir is needed in children than in adults to have a virological response, may be explained by the faster clearance of the drug in children than in adults. Table 1 illustrates that in order to obtain a similar AUC in both a child and an adult, a higher peak level and a lower trough level of indinavir can be observed in the child. Therefore, if trough levels are important to obtain a durable antiviral response (3,17,18), children will need higher AUC values than adults to obtain similar trough levels. It is remarkable that in this group of pediatric patients the success of combination antiretroviral therapy can be explained to a large extent by the plasma concentrations of only one of the three components of the drug regimen. A similar observation was found in adults [3]. Because the majority of children used zidovudine+lamivudine as the nucleoside background, and this may have influenced the observed relationship between indinavir AUC and virological outcome, it cannot be concluded from our data that the target AUC of indinavir is also valid for other nucleoside combinations.

There has been some concern that the risk of indinavir-induced nephrological toxicity (kidney stones, hematuria, flank pain) in children may be higher than in adults. As noted above, for that reason the maximum indinavir dose was originally reduced to 350 mg/m^2 in the first phase I/II trial of indinavir in the US (14). Because urological toxicity is the result of precipitation of indinavir crystals and this is correlated with the height of the plasma level of indinavir (5), one would expect a high incidence of urological toxicity in our study because most children used an indinavir dose that is even higher than 500mg/m^2. However, this was not the case. None of the children developed kidney stones, and only 3 children developed hematuria, which resolved after dose interruption. An explanation of this low incidence of urological toxicity in our children can not be given, but differences in the amount of fluid intake and climatological influences (higher temperatures in the US than in the Netherlands) are some of the possible reasons.

In conclusion, we found that the optimal dose of indinavir in children to obtain drug exposure similar to values observed in adult patients is 50 mg/kg MW q8h, which approximates 600mg/m^2 q8h. Using this dose, 70 percent of the children reach the goal of an undetectable viral

load (< 500 copies/mL) after 6 months of treatment, without the occurrence of serious adverse events. We also found that it would even be better to adjust the indinavir dose based on an $AUC_{0\text{-}8h}$ greater than 20 mg/L.h, because in that situation 100% of the children could be regarded as responders.

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Pharmacokinetics of indinavir and low-dose ritonavir twice daily in children with HIV-1 infection

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AIDS 2000;14:2209-10

Chapter 6

Abstract

Design: Retrospective: case serie. *Patients:* Four HIV-1 infected children with virological failure on treatment with a single protease inhibitor containing therapy regimen. *Interventions:* Patients were switched to an indinavir (600 mg/m^2 q12h) and low-dose ritonavir (100 mg/m^2 q12h) containing regimen. *Results:* With the exception of one patient (who had extremely high plasma levels of indinavir and ritonavir), adequate plasma levels of indinavir and ritonavir were achieved. In addition, an improved virologic response was observed in the two patients without extensive prior treatment. In one patient pyrexia of unknown origin occurred, subsided after indinavir/ritonavir therapy was discontinued and returned after rechallenge with indinavir/ritonavir. *Conclusion:* The combination of indinavir and ritonavir is currently the only protease inhibitor containing regimen without food restrictions. Our study shows that this regimen may be an alternative to the more complex and rigid single protease inhibitor containing medication schemes used in children with HIV-1 infection. However, in two of the four children drug related side-effects were observed. Controlled studies should be performed to evaluate the safety and efficacy of an indinavir/low-dose ritonavir regimen in the treatment of HIV-1 infected children.

Introduction

The use of highly active antiretroviral therapy (HAART) in children has only recently been reported and data are still limited, but HAART seems to be as successfully in children as in adults. The viral load becomes undetectable in a high percentage of the children and CD4 cell counts increase significantly. (1-8) Adherence to the rigid medication schemes is a major problem of HAART. Since the difficult medication regimens have to be continued for a prolonged period, the level of adherence may easily be reduced in time, which may lead to treatment failure. In children, in whom specific issues concerning compliance exists (i.e. evening medication in sleeping time, afternoon medication at school, forcing young children to eat), maintenance of compliance is further complicated by age-related differences in pharmacokinetics and pharmacodynamics. A higher metabolic clearance and an increased volume of distribution in young children necessitate frequent dosing, while monitoring of the plasma levels of the protease inhibitor is required to achieve adequate drug exposure. Adequate plasma levels of protease inhibitors are important because of the relation between low plasma concentrations and virological treatment failure. (9)

It is obvious that an urgent need exists for less frequent and less complex dosing schemes. The combination of indinavir with low-dose ritonavir is a possibility to accomplish a simplified BID regimen. Indinavir is rapidly metabolised by cytochrome P-450 3A isoenzymes (CYP3A), whereas ritonavir is a potent CYP3A inhibitor. This combination results in lower peak levels and higher trough levels of indinavir and reduces the intersubject variability in area under the curve (AUC) and trough levels in adults (8). In addition, the bioavailability is not affected by regular meals containing 32% of calories from fat, which is another practical benefit of this regimen. (8) We here report four children failing on single protease inhibitor containing regimens, who were switched to a combination of indinavir with low-dose ritonavir.

Methods

Four HIV-1 infected children of three different hospitals in whom protease inhibitor containing antiretroviral therapy failed, were switched to an indinavir and low-dose ritonavir containing regimen. Oral informed consent was obtained from the parents or care-givers.

Medication

Indinavir as single protease inhibitor (PI) is given as 500-600 mg/m^2 every 8 hour. (4, 10)When combined with ritonavir the same dose is given every 12 hour. The pediatric dose of ritonavir as single PI is 350 mg/m^2 every 12 hour; when given as low-dose with indinavir in adults the ritonavir dose is one sixth of the normal adult ritonavir dose (100 mg vs 600 mg). Because children have higher clearances of PI's, low dose ritonavir was given as one third of recommended pediatric dose : 100 mg/m^2 every 12 hour. No food-instructions were given. Indinavir was administered as 200 mg or 400 mg capsules or as syrup (10 mg/ml, developed in the university hospital Nijmegen, The Netherlands) in the case of patient 2, as this patient was not able to swallow capsules. The syrup was used within 14 days after preparation. Oral informed consent was obtained from the parents. Ritonavir was administered as syrup (80 mg/ml).

In two children that used indinavir before start of indinavir/ritonavir, pharmacokinetics of indinavir q8h were determined before adding ritonavir. Two weeks after start of indinavir and ritonavir, patients were admitted to the day-care unit of each hospital to determine the steady state pharmacokinetics of indinavir and ritonavir. Blood samples were collected just before ingestion of indinavir/ritonavir and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 hours post ingestion. Indinavir and ritonavir concentrations were determined by HPLC, as previously reported. (11)Pharmacokinetic parameters were calculated by non-compartmental methods. (12)

Case reports

Patients

In Table 1 baseline characteristics and medication are listed. In patient 1 plasma HIV-1 RNA load increased from undetectable to 1940 copies/ml after one year of treatment with indinavir, zidovudine and lamivudine. Two subsequent plasma HIV-1 RNA loads (three and five weeks later) were respectively 816 and 2630 copies/ml. Compliance seemed to be good. Because in this girl trough level (Cmin) of indinavir was low (0.03 mg/l, mean adults: 0.15) in the presence of an adequate peak level (Cmax) and area under the curve (AUC), ritonavir was added to achieve higher trough levels without increasing the peak level. The dosage of indinavir was 400mg (475 mg/m^2) q12h, the dosage of ritonavir was 80 mg (100 mg/m^2) q12h. Four weeks after the switch HIV-1 RNA decreased to below 500 copies/ml.

Patient 2 started at the age of 5 months with indinavir, zidovudine and lamivudine. In this patient AUC, peak level and trough level of indinavir were low (AUC: 7.41, Cmax: 3.78, Cmin: 0.06) and stayed low after increasing the dosage from 150 mg q8h (= 400 mg/m^2 q8h) to 200 mg (= 550 mg/m^2 q8h). Low-dose ritonavir was added to the regimen 3 months after HAART was initiated to improve pharmacokinetics of indinavir. The indinavir dosage was 200 mg (510 mg/m^2) q12h, the ritonavir dosage was 40 mg (100 mg/m^2) q12h.

Patients 3 and 4 failed respectively after two and three years on medication schemes without indinavir. In these patients all medication was switched to a ritonavir/indinavir containing regimen because of high HIV-1 RNA loads and extensive prior treatment. In patient 3 the ritonavir dose was: 100 mg (= 85 mg/m^2) q12h, indinavir 600 mg (= 510 mg/m2) q12h. In patient 4 indinavir and ritonavir were dosed as follows: indinavir 400 mg (= 490 mg/m2) q12h, ritonavir 80 mg (= 100 mg/m2) q12h.

Pharmacokinetics

Figure 1 shows the pharmacokinetic parameters of indinavir in the 4 children. In patient 1 and 2 pharmacokinetics of indinavir q8h are also presented (as patient 3 and 4 did not use indinavir before the start of indinavir/ritonavir, pharmacokinetics of indinavir q8h are not available for these patients). Table 1 shows the pharmacokinetic parameters of indinavir and ritonavir. With the exception of patient 3 adequate values of AUC, Cmax and Cmin of indinavir were achieved. Levels of ritonavir are comparable with levels of ritonavir measured in adults on an indinavir/ritonavir 800/100 mg BID regimen. (13) In one patient 3 extremely high levels of both indinavir and ritonavir were measured. We explored the possibility of accidently overdosing, but we could not find an obvious explanation of these high levels.

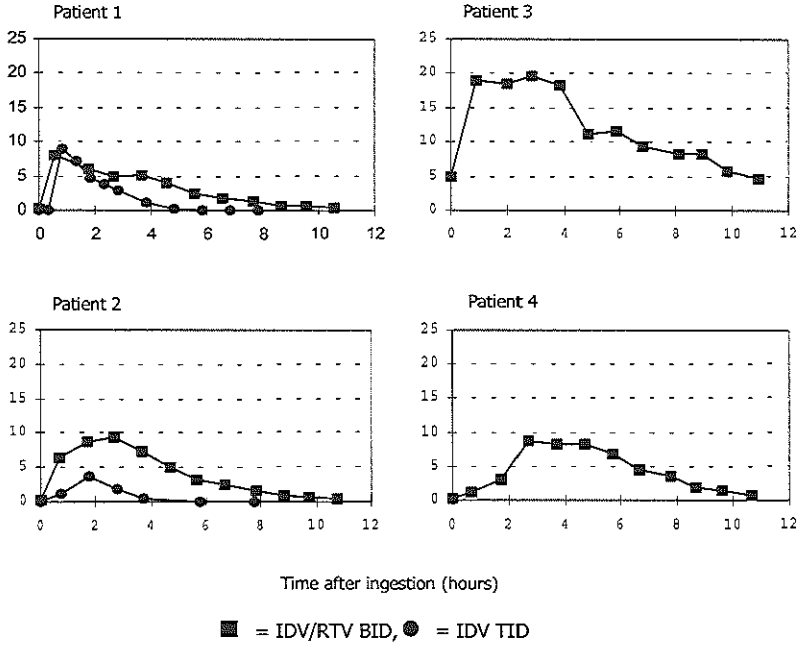

Figure 1: *Plasma concentrations of indinavir (mg/L).*

Table 1. Baseline characteristics and pharmacokinetic parameters

Patient	Age (yrs)	Gender	Medication History	Treatment before Switch	Treatment after Switch	Other Medication	Indinavir			Ritonavir		
							AUC 0-24 (mg/L.h)	Cmax (mg/L)	Cmin (mg/L)	AUC 0-12 (mg/L.h)	Cmax (mg/L)	Cmin (mg/L)
1	7	Female	ZDV (8 months)	IDV, ZDV, 3TC (14 months)	RTV, IDV, ZDV, 3TC	-	68.36	7.98	0.23	14.98	2.66	0.38
2	0.8	Female	-	IDV, ZDV, 3TC (3 months)	RTV, IDV, ZDV, 3TC	-	90.32	9.39	0.49	21.55	3.50	0.49
3	10.2	Female	ZDV, 3TC (56, 7 months)	NFV, d4T, NVP (18 months)	RTV, IDV, ddI	TMP-SMZ	265.64	19.57	4.63	88.72	12.46	4.22
4	6.7	Female	ZDV, 3TC (15, 5 months)	NFV, d4T, ddI (17, 34, 34 months)	RTV, IDV, NVP	-	94.88	8.65	0.31	19.58	3.21	0.53
Adults treated with IDV 800 mg q8h							57.36	9.40	0.15	19.58	3.21	0.53
Adults treated with IDV/RTV 800/100 mg q12h							100.40	8.30	1.25	11.79	2.06	0.56

IDV = indinavir RTV = ritonavir ZDV = zidovudine
NFV = nelfinavir NVP = nevirapine
3TC = lamivudine d4T = stavudine
ddI = didanosine TMP-SMZ = trimethoprim-sulfamethoxazol

Side effects

In 3 of the 4 patients side effects were seen. Patient 2 suffered from a rash, but the rash did not disappear until 2 months after switching indinavir/ritonavir into nelfinavir.

Patient 3, the patient with extremely high levels of both protease inhibitors suffered from abdominal pain, hematuria, fever and a rash on her back. These complaints disappeared after discontinuation of indinavir and ritonavir. Ultrasonography of the kidneys was unremarkable. Patient 4 complained of sleepiness during the day. Four months after the switch to indinavir and ritonavir pyrexia developed, showing a distinct pattern of spiking. Every six hours after taking indinavir, ritonavir and nevirapine temperature spiked to about 40°C. The girl also presented with intermittend vomiting, slight loss of appetite and night sweats. Blood cultures, throatswabs, urine malaria screen, mantoux test, CMV and toxoplasmosis serology, stool virology, cat scratch virology and ultrasonography of the abdomen were unremarkable. After discontinuation of the medication the temperatures disappeared and reappeared after rechallenge.

Discussion

Combining indinavir with low-dose ritonavir improves the pharmacokinetic profile of indinavir in adults, resulting in a BID medication scheme without the necessity for food restrictions.(14) This combination is currently the only protease inhibitor containing regimen without food restrictions. Because of specific issues concerning compliance in children, an urgent need for such a simplified regime for children exists.

We here report four children failing on protease inhibitor containing regimens, who were switched to indinavir combined with low-dose ritonavir.

Administration of indinavir (500 mg/m^2) combined with low dose ritonavir (100 mg/m^2) resulted in increased trough levels in the presence of peak levels comparable with indinavir 600 mg/m^2 q8h in three of the four children. The increased trough levels may result in more effective and more sustained virologic suppression, while peak levels are not increased and therefore side effects comparable to indinavir 600 mg/m^2 q8h may be expected. In one child (patient 3) extremely high values of all pharmacokinetic parameters for both drugs were found. An explanation of these high levels can not be given, but a mistake in the administered amount of medication seems unlikely. An extremely low metabolic clearance is a possible explanation.

Hsu et al describe pharmocokinetics of different regimens containing a combination of indinavir and ritonavir. (14) The combination of indinavir/ritonavir 400/400 mg has the lowest peak levels and may therefore have a lower incidence of urological complications (15), while the trough level is almost 3 times higher than with 800 mg q8h, which is important for viral suppression. As it is very difficult to make children drink ritonavir, because of its nasty taste, the proportion of indinavir and ritonavir 500-600/100 mg/m2 (800/100mg in adults) as described by Burger et al (13) was chosen to reduce the amount of ritonavir liquid.

Side effects of this dual protease inhibitor containing regimen seem to be frequent as in three of the four patients side effects were observed. However, with exception of pyrexia of unknown origin in patient 4, the only severe side effects were seen in the patient with the extremely high levels of indinavir and ritonavir. The urological complaints of this patient may be explained by the association between high levels of indinavir and urological complications. (15) Patient 2 suffered from a rash.

However, the rash patient 2 suffered from is unlikely to be due to indinavir/ritonavir since the rash did not disappear after switching indinavir/ritonavir into nelfinavir.

In conclusion, our data show that an indinavir/ritonavir 500-600/100 mg/m^2 BID containing regimen may be a good alternative to the more complex and rigid single protease inhibitor containing medication schemes from a pharmacological point of view. However, the substantial toxicity in this small number of children needs further investigations. Controlled studies should be performed to evaluate the safety and efficacy of an indinavir/ritonavir BID regimen in children.

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Therapeutic drug monitoring of indinavir and nelfinavir to assess adherence to therapy in HIV-1 infected children

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Chapter 7

Abstract

Introduction: Adherence to highly active antiretroviral therapy (HAART) is required to obtain an optimal long-term virological response rate of HIV-1 infected children. An accurate assessment of the level of compliance is complicated by the limitations of the current methods. Plasma concentrations of protease inhibitors (PIs) outside the limits of the reference values indicate non-adherence to antiretroviral therapy in adults. During a two-year follow-up period we studied routinely taken plasma protease inhibitor levels to assess compliance to antiretroviral therapy in HIV-1 infected children. *Methods:* In 40 children (age: 3 months to 18 years) blood samples were taken at regular outpatient visits every 12 weeks after the start of HAART and analyzed for plasma levels of indinavir or nelfinavir by HPLC and for plasma HIV-1 RNA load. The percentage of samples fulfilling the criteria for compliance was assessed for each child using three different methods. For each sample a concentration ratio was calculated by dividing the concentration in that sample by the time adjusted population value. According to method 1 concentration ratios below or above concentration ratio limits (CORALs) of population data obtained in adults were analyzed. This method is highly indicative of non-compliance in adults. Since many children have high PI levels method 2 evaluated plasma samples of PIs using only the lower CORAL. According to method 3 only children with plasma samples below the limit of quantification (0.04 mg/L) were considered non-compliant. Differences in compliance rate between virological responders and virological non-responders and between the two protease inhibitors were analyzed. The cumulative incidence of HIV-1 RNA levels >500 (copies/ml) in children was calculated. *Results:* Thirty-one children started treatment with indinavir and nine children with nelfinavir. The median compliance rates (interquartile range (IQR)) for indinavir as determined by methods 1, 2, and 3 were 54 (25-69)%, 67 (50-92)% and 80 (63-100)% respectively. For nelfinavir median (IQR) compliance rates of 60 (39-75)%, 100 (67-100)% and 100 (100-100)% were observed. Compliance rates calculated with method 2 were significantly higher in virological responders (p=0.04). Compliance rates calculated with methods 2 and 3 were significantly lower in children using indinavir compared with those using nelfinavir (p=0.02 and p=0.02, respectively). *Conclusion:* PI plasma levels below the lower CORAL are highly indicative of non-compliance for children on indinavir and nelfinavir and may thus be a useful method for the assessment of non-adherence to antiretroviral therapy in children.

Introduction

The institution of highly active antiretroviral therapy (HAART) in HIV-1 infected adults and children has led to increasingly complex drug regimens. Adherence is required to obtain optimal suppression of the virus, which is necessary for an optimal long-term virological response. (1-3) Poor compliance is associated with several factors including: age (with adolescents being less compliant), the number of different medications, the number of doses of the medication, the extent to which the regimen interferes with the patient's life, the occurrence of side-effects in asymptomatic patients and longer duration of the disease. (4-10) All factors that are associated with poor compliance are also present in HIV infected children. Therefore optimalization of the use of HAART in children poses a challenge for physicians involved in the treatment of children with HIV/AIDS.

Overall compliance rates in adults are 75% versus only 58% in children. (11-14) The high percentage of children who do not adhere to antiretroviral therapy and the subsequent risk for

virological failure and long-term clinical failure underscores the importance of the development of methods to predict poor adherence. This may subsequently contribute to early intervention and optimalization of the antiretroviral regimen. The analysis of the level of adherence is complicated by the limitations of the current methods. Self-reported measures, electronic monitoring systems (Medication Event Monitoring System (MEMS)), pill counts, blood levels of protease inhibitors and refill history have been used. (11-16) However, all of them have their limitations. Recently Hugen et al. reported that plasma concentrations of protease inhibitors in adults outside the limits of a reference population strongly suggest non-compliance. (17) This method is objective, easy to perform and can be an attractive way to assess non-compliance in combination with other objective measures. This method has not yet been applied in children and is potentially complicated by the large interindividual differences in the pharmacokinetics of protease inhibitors in children. (18, 19) We here report the results of a study to assess adherence to antiretroviral therapy in HIV-1 infected children by means of therapeutic drug monitoring of nelfinavir and indinavir and the association between plasma levels and the virological response rate.

Methods

Inclusion and exclusion criteria

PI naive children between the ages of 3 months and 18 years were eligible for enrolment in five participating centers between April 1997 and May 2000. HIV-1 infected children with an HIV-1 viral load of more than 5000 copies/ml and/or a CD4 cell count below the age-specific reference values were included. (20)

Methods

The study was approved by the medical ethical committees of all the participating centers. Written informed consent was obtained from parents or legal guardians. Blood samples were taken after a median (interquartile range (IQR)) time of 3.8 (3-5) hours after the ingestion of the protease inhibitor, at regular outpatient visits every 12 weeks after the start of HAART and stored at -20 °C. Time of blood sampling and time of the last administration of the protease inhibitor was recorded in the case record form. The concentrations of nelfinavir and indinavir plasma samples were analyzed by HPLC. (21, 22) Children were excluded when less than three samples were available. Plasma HIV-1 RNA quantitation was analyzed by a polymerase chain reaction assay (Roche Amplicor HIV-1 monitor test version 1.5) at the same timepoints as plasma drug levels.

Medication and pharmacokinetics

Medication was administered in the following doses: indinavir 400 mg/m^2 q8h or nelfinavir 30 mg/kg q8h, zidovudine 120 mg/m^2 q8h, lamivudine 4 mg/kg q12h. A day to day medication scheme including the times with food restrictions was given.

At week 4, patients were admitted to the day-care unit to determine the steady state pharmacokinetics of indinavir or nelfinavir. When a dosage adjustment of indinavir or nelfinavir was necessary to normalize the area under the curve-concentration (AUC) curve to adult values (indinavir: 10-30 mg/L*hr, nelfinavir: 13-20 mg/L*hr), this procedure was repeated.

Statistical Analyses

The percentage of samples fulfilling the criteria for non-adherence to antiretroviral therapy was assessed for each child using three different methods. Plasma levels of protease inhibitors in children were plotted in a concentration-time curve, which was derived from steady state population pharmacokinetics in adults. For each sample a concentration ratio was calculated by dividing the concentration in that sample by the time adjusted population value. According to method 1 concentration ratios below or above concentration ratio limits (CORALs), consisting of the 5th and 95th percentile of population data obtained in adults, were highly indicative of non-compliance. For indinavir 800 mg q8h, CORALs were <0.23 and >3.3 mg/L. For nelfinavir 750 mg q8h and 1250 mg q12h, CORALs were <0.36 and 2.1 (mg/L). (17) Since many children have high PI levels method 2 evaluated plasma samples of PIs using only the lower CORAL. Method 3 was based on a more traditional approach in which only children with plasma samples below the limit of quantification (0.04 mg/L) were considered to be non-compliant. Percentages were calculated by dividing the number of plasma levels within the limits defined by the 3 different methods by the number of visits. Virological responders were defined as children who had an HIV-1 RNA level below the limit of detection of 500 copies/ml at week 12 and during follow-up.

SPSS 9.0 (SPSS, Chicago) was used for statistical analysis. Differences in the compliance rates between virological responders and virological non-responders and between compliance rates and the two protease inhibitors were analyzed using the Mann Whitney U test (p-values are 2-tailed). The cumulative incidence of HIV-1 RNA levels >500 (copies/ml) in children was calculated by means of Kaplan-Meier analysis. The influence of a compliance rate <75% and ≥75% was determined with the logrank test. The percentage of 75% was chosen, because Watson et al. observed a higher virological failure rate in children who were non-adherent (defined as <75% of antiretroviral therapy prescriptions filled). (12)

Results

Patient characteristics

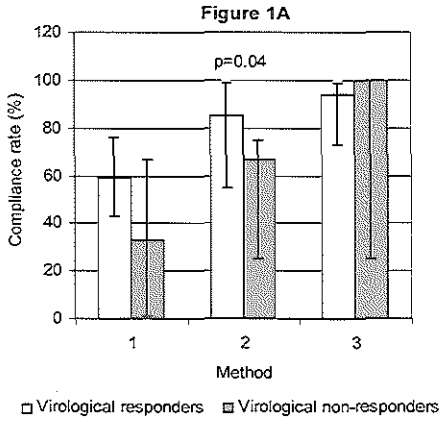
Forty children with a median (range) age of 5.4 (0.28-16.3) and a median (range) baseline plasma HIV-1 RNA of 136,385 (2,680-2,480,000) were included. Thirty-one children started treatment with indinavir and nine children with nelfinavir. The median number of samples per patient (range) was 6 (3-11) in the indinavir group and 4 (3-9) in the nelfinavir group. Twenty-two children were classified as virological responders and 18 as non-responders.

Compliance rates

Methods 1 (concentration ratios below or above CORALs), 2 (below lower CORAL) and 3 (below limit of quantification) resulted in median compliance rates (IQR) for indinavir of 54 (25-69)%, 67 (50-92)% and 80 (63-100)% respectively. The median (IQR) compliance rates for nelfinavir were 60 (39-75)%, 100 (67-100)% and 100 (100-100)%. The median percentages were highly variable between methods 1, 2 and 3.

Relation between compliance and virological response

In Figure 1A the compliance rates calculated with the three different methods in virological responders and non-responders are depicted. No difference between virological responders and non-responders in compliance rate was observed when analyzed with method 3 (p=0.6). However, compliance rates calculated with method 1 showed a trend to a higher compliance rate in virological responders (p=0.07) and compliance rates calculated with method 2 were significantly higher in virological responders. In virological responders a median (IQR) compliance rate of 85 (55-100)% was observed with method 2, whereas the median (IQR) compliance rate in non-responders was 67 (25-75)% (p=0.04).



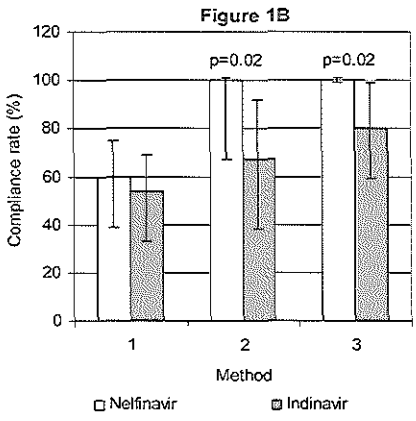


Figure 1A: *The compliance rates calculated with the three different methods in virological responders and non-responders are depicted. Error bars represent the interquartile ranges.*

Figure 1B: *The compliance rates calculated with the three different methods in children using indinavir and in children using nelfinavir. Error bars represent the interquartile ranges.*

Figure 2 A, B and C show the cumulative incidences of patients with an HIV-1 RNA load > 500 (copies/ml) in children with a compliance rate <75% and ≥75% as calculated with methods 1, 2 and 3. Children with a compliance rate <75% more frequently (method 1, 2 and 3: p=0.06, p=0.01 and p=0.2 respectively) had an HIV-1 RNA load >500 (copies/ml).

Compliance rate and influencing factors

Figure 1B presents the compliance rates calculated with the three different methods in children using indinavir and in those using nelfinavir. Compliance rates calculated with methods 2 and 3 were significantly lower in children using indinavir compared with those using nelfinavir (p=0.02 and p=0.02, respectively).

Age, ethnicity, prior treatment with antiretroviral medication and CDC classification stage were not related with compliance rates.

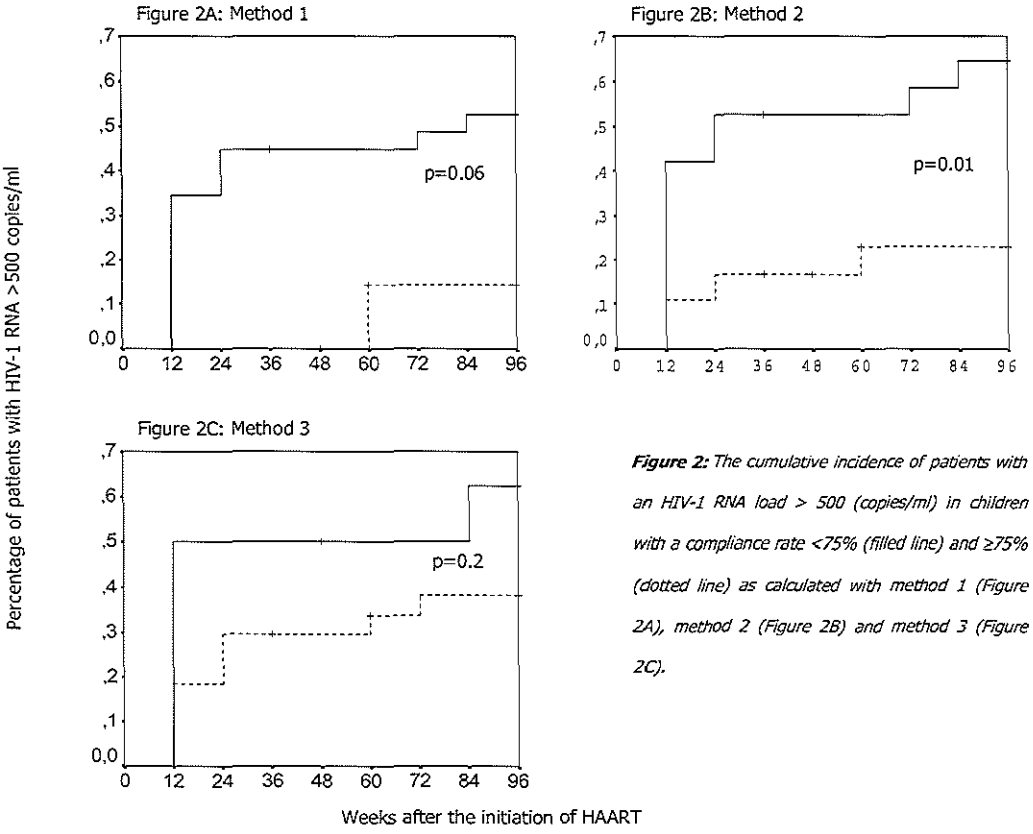


Figure 2: *The cumulative incidence of patients with an HIV-1 RNA load > 500 (copies/ml) in children with a compliance rate <75% (filled line) and ≥75% (dotted line) as calculated with method 1 (Figure 2A), method 2 (Figure 2B) and method 3 (Figure 2C).*

Discussion

We studied the value of the measurement of the routinely determined plasma concentrations of indinavir and nelfinavir. The percentage of samples fulfilling the criteria for compliance was assessed for each child using three different methods. For each sample a concentration ratio was calculated by dividing the concentration in that sample by the time adjusted population value. According to method 1 concentration ratios below or above concentration ratio limits (CORALs) were defined as non-compliant. Since many children have high PI levels method 2 evaluated plasma samples of PIs using only the lower CORAL. According to method 3 only children with plasma samples below the limit of quantification were considered non-compliant.

The compliance rates were highly variable between the 3 methods. When method 1 was used 22 of 31 (71%) and 7 of 9 (78%) of the children using indinavir and nelfinavir were indicated as non-compliant respectively. However, when method 2 was applied the numbers of non-adherent children were reduced to 16 of 31 (52%) and 3 of 9 (33%). This reduction in the non-compliance rate may be explained by the deletion of one limit and by the higher peak concentrations of protease inhibitors that are observed in children. (18, 19) Compliant children with higher plasma

concentrations of the protease inhibitor due to pharmacokinetic differences between children and adults are considered to be non-compliant in method 1 whereas according to method 2 these children would be considered compliant. Since administering the medication too late can also cause higher plasma concentrations, in method 2 imperfect compliance (administration of the drugs, but not in time) is considered as good adherence. Therefore this may result in an underestimation of non-compliance. Method 3 also underestimates non-compliance, but a concentration below the limit of quantification of the protease inhibitor is highly indicative of non-compliance.

A relation between adherence rate and virological response rate has been demonstrated in both HIV-1 infected adults and children treated with HAART. (11, 12) In our study a difference between the adherence rate to antiretroviral therapy in virological responders and non-responders was observed with method 2 and a trend to a relation was observed with method 1. This indicates that the determination of plasma levels of the protease inhibitors indinavir and nelfinavir followed by calculation as concentration ratio of the lower CORALs (method 2) in children may be a useful measurement for the assessment of compliance in children. The absence of a significant relation between virological response and non-compliance according to method 3 may be due to the limited number of children and due to still present detectable plasma levels of nelfinavir after 2 missed doses. (17) Another explanation might be a "white coat effect", which means that compliance may be better at the day the hospital is visited. Whether the measurement of plasma concentration levels below the limit of quantification has additional value remains to be evaluated in a study with a larger number of children.

Children treated with nelfinavir showed significantly higher compliance rates compared to children treated with indinavir. For method 3 this may be explained by the fact that it takes more missed doses of nelfinavir (2.3) than of indinavir (1.3) before plasma levels become undetectable. (17) The difference observed with method 2 may be explained by a real difference in compliance rates between the two PI's.

We conclude that the compliance rates calculated as the percentage of samples that fulfilled the criteria for compliance using only the the lower CORALs (consisting of the 5th percentile of population data obtained in adults) of indinavir or nelfinavir in children and virological response are associated. This method may therefore be a useful measurement for the assessment of non-compliance in children.

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Part III

Clinical, virological, and immunological aspects of highly active antiretroviral therapy in HIV-1 infected children

Efficacy of highly active antiretroviral therapy in HIV-1 infected children

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Chapter 8

Abstract

Introduction: Although the reduction in HIV-1-related deaths is similar in adults and children, the extent of the changes in two important surrogate markers (HIV-1 RNA levels and CD4+ T-cell counts) differs widely. In most pediatric studies virological response rates to highly active antiretroviral therapy (HAART) are inferior to those in adults. This review provides an overview of the pediatric clinical studies using HAART and seeks to improve the understanding of factors, which may contribute to success or failure of HAART in children. *Methods:* An overview of all current articles on pediatric clinical trials using HAART is provided. Twenty-three papers were available. HIV-1 RNA loads and CD4+ T-cell counts were used as primary outcome measures. *Results:* Virological response rates were highly variable both between the different antiretroviral drugs but also between different studies using the same medication. Four studies in which dosages of the administrated PI were adjusted after pharmacokinetic evaluation had superior virological response rates compared to those in which fixed dosages were used. Immunological response rates were more uniform than virological responses. In almost all studies increases of CD4+ T-cell counts are reported independent of the extent of the virological response. Side-effects of HAART were generally mild, transient and of gastro-intestinal origin. Significant percentages of patients with serum lipid abnormalities were reported in three pediatric studies. However, signs of clinical lipodystrophy were not observed. *Conclusion:* The inferior virological response rates, which have been reported in HIV-1 infected children treated with HAART form a reflection of the challenges which are encountered in the treatment of these children. Difficulties with adherence and with the pharmacokinetics of protease inhibitors in children require an intensive, child adjusted approach. A practical approach to therapy in institutions without tertiary top-reference care facilities may be induction therapy with a lopinavir containing regimen (lacking a need for therapeutic drug monitoring) to reduce high viral load levels followed by an easily tolerated maintenance regimen for example containing abacavir or nevirapine.

Introduction

Since the introduction of highly active antiretroviral therapy (HAART) a reduction in the rate of progression of AIDS and HIV-1-related deaths has been observed among adults living in the Western world. (1, 2) The effectiveness of HAART in infants and children to reduce HIV-1-related deaths is at least similar, or even greater than that observed in adults. (1)

The measurement of two surrogate markers, HIV-1 RNA levels and CD4+ T-cell counts, has become the basis for the prediction of clinical, virological and immunological responses in both HIV-1 infected adults and children treated with HAART. (2, 3) Although the reduction in HIV-1-related deaths is similar in adults and children, the extent of the changes in these parameters differs widely. In most pediatric studies virological response rates to HAART are inferior to those in adults. (4-12) Since virological suppression is associated with long-term success of HAART, this may have major implications for the future health of these children. (13-15)

The institution of optimal treatment regimens in HIV-1 infected children poses a enormous challenge. First, large interindividual differences of pharmacokinetics of antiretroviral drugs, especially of protease inhibitors, complicate optimal dosing of antiretroviral drugs in children. Secondly, it is difficult to maintain adherence to combination therapy during many years. Problems such as the intake of evening medication during sleeping time or afternoon medication during

school, unwillingness of young children and adolescents to take medication, poor palatability and side-effects of medication have to be dealt with. Thirdly, different viral dynamics may complicate optimal suppression of HIV-1. Viral load reduction in children following the introduction of HAART has a slower phase II decay rate in children in comparison with adults. (16) Baseline viral loads in children are higher, which may be a barrier to reach undetectable viral loads. (17-19)

Despite the difficulties in the treatment of HIV-1 infected children and the poor virological responses in many studies, it has become clear that a similar rate of suppression of HIV-1 replication may be obtained in children during the first year of treatment as compared to that in adults. (20-26) The reasons for the highly variable therapy results in children are not well understood. This review provides an overview of the pediatric clinical studies using HAART and seeks to improve the understanding of factors, which may contribute to success or failure of HAART in children.

Methods

An overview of all current articles on pediatric clinical trials using HAART is provided. Search strategy: The Pubmed database (www.ncbi.nlm.nih.gov) was used to search for articles on pediatric clinical trials using HAART.

Selection criteria: articles not written in English were excluded. No selection on date was made. Articles with text word combinations: HIV-1 and children, HIV-1 infection and children, ritonavir and children, indinavir and children, nelfinavir and children, saquinavir and children, nevirapine and children and abacavir and children were selected.

Twenty-three papers were available. HIV-1 RNA loads and CD4+ T-cell counts were used as primary outcome measures.

Ritonavir

In March 1996, ritonavir (RTV) was the first protease inhibitor approved by the US Food and Drug Administration (FDA) for the treatment of HIV-1 infected children (age 2 to 16 years). RTV has been available in a pediatric formulation (liquid) and leads when used as a single agent to a marked and rapid decline of plasma HIV-1 RNA levels. (27) However, the HIV-1 RNA levels gradually return toward baseline values after a few weeks of treatment, presumably because of the development of resistance. Virological response rates were sustained for a 24-week period after administration of the highest dose (400 mg/m^2 q12h). (27) The virological response rates of combinations of RTV with one or two nucleosides were also modest with 16%, 14%, 32% and 42% below 400 copies HIV-1 RNA/ml after 24, 52, 72 and 48 weeks respectively. (10, 11, 22, 27) CD4+ T-cells significantly increased in most children irrespective of the extent of the virological suppression. Toxicity by RTV consisted mainly of gastro-intestinal symptoms (nausea, vomiting). These symptoms were frequently reported as mild and transient although in 4-23% more severe gastro-intestinal symptoms, fever or rash were observed. (10, 11, 22, 27) Thuret et al. reported an increase in the serum levels of triglycerides and cholesterol of 33% and 61% respectively after at least 12 months of therapy, although no clinical signs of lipodystrophy were reported. (11) In Table 1 the study results with RTV are summarized. The poor virological efficacy in children, the serious side-effects which are seen in a

high percentage of the children and the poor taste of the liquid formulation, form in important disadvantages in the use of this PI.

Indinavir

In March 1996 the protease inhibitor indinavir (IDV) was registrated by the FDA for use in HIV-1 infected adults. Although IDV thus far only has been available in a capsule formula, it is possible to dissolve IDV in water for use in infants and young children. (24) Indinavir has to be administered to a fasting child at least one hour before or two hours after the intake of food.
Virological response rates of IDV combination therapy with two NRTI's differ widely. Mueller et al. reported that 6% of their 54 patients had an HIV-1 RNA load below the detection limit of 200 copies/ml after 16 weeks. (28) After 96 weeks only 4 (12%) of the 33 patients that completed a follow-up of 96 weeks. (29) This very low response rate was probably due to the administration of IDV as monotherapy during the first 12 weeks. Van Rossum et al. and Vigano et al. reported higher virological response rates: 70% <500 copies/ml and 87% <400 copies/ml after 24 and 72 weeks respectively. (20, 24) In all these studies irrespective of the virological response rate immunological improvement was reported. Renal side-effects by IDV due to crystallisation of indinavir in the kidney were reported in 11 to 80% of the patients and varied from crystalluria and hematuria to nephrolithiasis. Nephrolithiasis was documented in 2 to 28% of the children. (4, 5, 7-9, 20, 24, 28, 29) Gastro-intestinal symptoms formed the other major side-effects of indinavir. (4, 5, 7-9, 20, 24, 28, 29)

Van Rossum et al. demonstrated that the administration of IDV with low-dose ritonavir in a twice daily regimen results in a higher AUC and in higher trough levels of indinavir independent of food intake. (30) Pediatric studies on the efficacy of a twice daily regimen with indinavir and low-dose ritonavir in children are ongoing. In Table 2 the pediatric studies with IDV are summarized. The results of these studies differ widely. The use of IDV has been associated with a virological response rate which is comparable to that obtained in studies with this protease inhibitor in adults. However, nephrotoxicity is a major side-effect of IDV. Since this side-effect is associated with high drug levels of IDV, pharmacokinetic monitoring is necessary to reduce the risk for nephrotoxicity. *(31)* Urinalysis should be routinely performed in children treated with IDV. Frequent dosing and food restrictions may result in poorer adherence. The combination of IDV and low-dose RTV results in a simplified regimen and should therefore be further explored in children.

Nelfinavir

Nelfinavir (NFV) was the third protease inhibitor approved by the FDA for use in HIV-1 infected children in March 1997. NFV has been available in a pediatric formulation (powder 50 mg/g). NFV has to be administered with food to guarantee an optimal absorption. NFV has been studied in combination with two NRTI's and with two NRTI's and a NNRTI. (21, 23, 32) The virological response rates in children treated with a combination of NFV and 2 NRTI's vary from 69% <400 copies/ml and 44% <50 copies/ml in an intention to treat analysis (23) to 44% <400 copies/ml (33).

Table 1: Pediatric studies using Ritonavir (RTV)

Author	Regimen	RTV dose (q12h)	Inclusion criteria	Number of patients	Follow – up (weeks)	Outcome HIV-1 RNA	Outcome CD4+ T-cells	Toxicity	Reference
Mueller et al.	RTV+ after 12 weeks ZDV and/or ddI	250, 300, 350 or 400 mg/m^2	Age 0.5-18 years, Naive, CDC stage 2, 3, B or C	48	24	↓ 2 $\log_{10}$ cop/ml (with 400 mg/m^2) 16% <200 cop/ml	Med ↑ 79 cells/mm^3	Mild and transient nausea, diarrhea, abdominal pain (98%)	(27)
Pelton et al.	RTV+d4T or ZDV with of without 3TC	350 mg/m^2	PI naive, HIV-1 RNA> 50,000 copies/ml and/or severely ↓ CD4+ cell count	43	52	↓ 1.69 $\log_{10}$ cop/ml 14% <400 cop/ml	Med ↑ 588 cells/mm^3	Vomiting, ↑ liver enzymes (9%) necessitating discontiuation	(10)
Thuret et al.	RTV+ZDV or d4T+3TC	350-400 mg/m^2	PI naive	22	72	↓ 1.5 $\log_{10}$ cop/ml 32% <400 cop/ml	Med ↑ 472 cells/mm^3	4% gastro-intestinal symptoms necessitating discontiuation ↑ triglycerides or cholesterol in 33% and 61% respectively	(11)
Nachmann et al.	RTV+ZDV+3TC or d4T/RTV or AZT/3TC	350 mg/m^2	Age 2-17 years, PI naive, Clinically stable	297	48	42% <400 cop/ml (RTV/ZDV/3TC) and 27% (RTV/d4T)	Med ↑ 818 cells/mm^3 =33% of total T-cells (RTV/ZDV/3TC) Med ↑ 767 cells/mm^3 =29% of total T-cells (RTV/d4T)	Grade 3: 23% (RTV/d4T) and 17% (RTV/ZDV/3TC): nausea, vomiting, rash, fever, neutropenia	(22)

Table 2: Pediatric studies using Indinavir (IDV)

Author	Regimen	IDV dose (q8h)	Inclusion criteria	Number of patients	Follow - up (weeks)	Outcome HIV-1 RNA	Outcome CD4+ T-cells	Toxicity	Reference
Rutstein et al.	IDV+2 NRTI's (n=19) or RTV+2 NRTIs (n=9)	480 (range: 333-571) mg/m^2	PI naive	28 (19 on IDV)	24	↓ 1.99 $\log_{10}$ cop/ml (IDV =RTV) 25% <400 cop/ml	Med ↑ 202 cells/mm^3	IDV: 44% renal side effects. 28% nephrolitiasis	(4)
Melvin et al.	IDV+2 NRTI's (n=5) or RTV+2 NRTIs (n=4)	500 mg/m^2	PI naive	9 (5 on IDV)	28-52	↓ 1.7 $\log_{10}$ cop/ml 22% <400 cop/ml	Med ↑ 499 cells/mm^3	80% (40% when misdosage is excluded): renal complications	(5)
Monpoux et al.	IDV, d4T, 3TC	500 mg/m^2	Severe immunodeficiency, High viral load, clinically stable PI naive	7	24	↓ 0.6 $\log_{10}$ cop/ml 14% <400 cop/ml	Med ↑ 132 cells/mm^3	Vomiting (n=1), mild ↑ billrubin	(9)
Mueller et al.	IDV+ after 12 weeks: ZDV+3TC	250 mg/m^2 or 350 mg/m^2 or 500 mg/m^2	Age 6 months-18 years, CDC stage B, C, 1, 2, Normal hematological and chemistry parameters, Clinically stable PI naive	54	16	↓ 0.07 $\log_{10}$ cop/ml (250 and 350 mg/m^2), ↓ 0.76 $\log_{10}$ cop/ml (500 mg/m^2), 6% <200 cop/ml (on triple)	Med ↑ 60 cells/mm^3	Generally well tolerated. 13% hematuria, 2% nephrolithiasis	(28)
Kline et al.	IDV+d4T+ddI	500 mg/m^2	Symptomatic HIV disease or immunosuppression ≥ 1 year NRTI's	12	48	↓ 2 $\log_{10}$ cop/ml	Med ↑ 317 cells/mm^3	33% nausea, vomiting 50% crystalluria. 42% hematuria, 80% pyuria, 17% transient jaundice	(8)
Wintergerst et al.	IDV+ZDV+3TC or IDV+d4T+3TC	500 mg/m^2	Age <18 years PI naive	15	24	↓ 1.6 $\log_{10}$ cop/ml 40% <400 cop/ml	Med ↑ 101% above baseline	NA	(7)

Vigano et al.	IDV+d4T+3TC	500 mg/m^2	Symptomatic HIV disease, Immunosuppression and prior NRTI use PI naive	25	72	87% <400 cop/ml	Med ↑ 360 cells/mm^3 =10% of total T-cells (at month 12)	24% renal symptoms 4% transient jaundice	(20)
Van Rossum et al.	IDV+ZDV+3TC	400, 500, 600 or 660 mg/m^2	PI naive, HIV-1 RNA >5000 cop/ml Immunosuppression Age 3 months-18 years	28	24	70% <500 cop/ml 48% <40 cop/ml	Med ↑ 100 cells/mm^3 =27% in relation to normal values	41% side-effects, mainly gastro-intestinal Renal symptoms in 11%	(24)
Jankelevich et al.	IDV+ after 12 weeks: ZDV+3TC	250, 350, or 500 mg/m^2 After week 58: 350 mg/m^2	Age 6 months-18 years, CDC stage B, C, 1, 2, Normal hematological and chemistry parameters, Clinically stable PI naive	33	96	↓ 0.74 log_{10} cop/ml 12% <200 cop/ml (on triple)	Med ↑ 199 cells/mm^3	NA	(29)

Table 3: Pediatric studies using Nelfinavir (NFV)

Author	Regimen	NFV dose (q8h)	Inclusion criteria	Number of patients	Follow - up (weeks)	Outcome HIV-1 RNA	Outcome CD4+ T-cells	Toxicity	Reference
Krogstad et al.	NFV+2 NRTIs	20-30 mg/kg	Age 0-13 years, PI naive, Clinically stable	62	54 (median 42)	71% ≥ ↓ 0.7 $\log_{10}$ cop/ml	Remained constant	25% grade 1 or 2 transient diarrhoea Grade 1 nausea, flatulence, anorexia, epistaxis, fever, neutropenia, abdominal pain, anemia and rash	(32)
Funk et al.	NFV+ZDV+3TC or NFV+d4T+ddI	20-30 mg/kg	Antiretroviral naive, CDC B, C, 2 or 3, HIV-1 RNA >20,000 (> 2 years) HIV-1 RNA >100,000 (< 2 years)	16	48	Med ↓ 2.8 $\log_{10}$ cop/ml 69% <400 cop/ml (ITT) 44% <50 cop/ml (ITT)	Med ↑ 157 cells/mm^3 =33% of total T-cells	Initial transient diarrhoea, lack of concentration and rash. Transient or persistent ↑ triglycerides or cholesterol in 50% and 31% respectively	(23)
Starr et al.	NFV+EFV+2NRTIs	20-30 mg/kg	Age < 16 years, HIV-1 RNA >400 cop/ml, PI and NNRTI naive, Ability to swallow capsules	48	57	Med ↓ 2.7 $\log_{10}$ cop/ml 81% <400 cop/ml (AT) 61% <400 cop/ml (ITT) 70% <50 cop/ml (AT) 53% <50 cop/ml (ITT)	Med ↑ 74 cells/mm^3 =3% of total T-cells	25% moderate, 9% severe , 2% life threatening. Most common:rash, diarhhea, neutropenia, biochemical abnormalities	(21)

Wiznia et al.	NFV+d4T+3TC (!) or NFV+d4T+NVP (2) or NFV+d4T+3TC+ NVP (3) or RTV+d4T+NVP (4)	30 mg/kg 55 mg/kg q12h (n=12)	Age 4 months-17 years, Stable CDC 1 or 2 cat. PI, NNRTI, d4T, 3TC naive, Clinically stable	193	24	1: 44% <400 cop/ml 2: 50% <400 cop/ml 3: 63% <400 cop/ml 4: 46% <400 cop/ml	1: Med ↑ 105 cells/mm^3 2: Med ↑ 87 cells/mm^3 3: Med ↑ 294 cells/mm^3 4: Med ↑ 254 cells/mm^3	77% ≥grade 2 Most comon: rash, nausea, vomiting, fever	(33)

Table 4: Pediatric studies using Saquinavir (SQV)

Author	Regimen	PI Dose	Inclusion criteria	Number of patients	Follow - up (weeks)	Outcome HIV-1 RNA	Outcome CD4+ T-cells	Toxicity	Reference
Hoffmann et al.	A: SQV+RTV+≥1 NRTI (n=6) or B: SQV+NFV+≥1 NRTI (n=5)	SQV+RTV: 15-30mg/kg +250-400 mg/m^2 q12h SQV+NFV: 15-30mg/kg +30-35 mg/kg q8h	Failure of prior therapy including at least 1 PI, Age <14 years	11	24	A: Med ↓ 1.4 log_{10} cop/ml 20% <200 cop/ml (AT) 0% <50 cop/ml B: Med ↓ 0.2 log_{10} cop/ml 0% <200 cop/ml (AT)	A: Med ↑ 23% above baseline = Med ↑ 281 cells/mm^3 B: Med ↑ 7% above baseline = Med ↑ 3 cells/mm^3	45% grade 1 or 2 diarrhea 91% ↑increase of triglycerides above normal 55% mild increase lever enzymes	(35)
Kline et al.	1: SQV-SGC+2NRTIs 2: SQV-SGC+NFV+2NRTIs	1: SQV-SGC 33mg/kg q8h 2: SQV-SGC 33mg/kg + NFV 30mg/kg q8h	Age 3-16 years, PI naive, Naive to ≥1 prescribed NRTI CDC stage A, B, C, 1 or 2, Normal hematological and biochemical parameters	1: 14 2: 13	1: 72 2: 48	1: Med ↓2.12 log_{10} cop/ml 36% <50 cop/ml 2: Med ↓2.58 log_{10} cop/ml 62% <50 cop/ml	1: Med ↑292 cells/mm^3 2: Med ↑154 cells/mm^3	No differences between 1 and 2. Generally mild: diarrhea (36%), abdominal discomfort (16%), headache (16%)	(36)

Table 5: Pediatric studies using Nevirapine (NVP)

Author	Regimen	NVP Dose	Inclusion criteria	Number of patients	Follow - up (weeks)	Outcome HIV-1 RNA	Outcome CD4+ T-cells	Toxicity	Reference
Luzuriaga et al.	NVP	1.120 mg/m^2/day or 2. 240 mg/m^2/day or 3.120 mg/m^2/day first 14 days followed by 240 mg/m^2/day or 4. <9years: 120 mg/m^2/day first 14 days followed by 400 mg/m^2/day	Age 2 months-18 years, Immunosuppression, CDC stage A, B, C Prior antiretroviral treatment <6 weeks	21	8	NA	43% ≥5% increase	5% Rash	(40)
Luzuriaga et al.	NVP+ZDV +3TC	120 mg/m^2/day first 28 days followed by 400 mg/m^2/day	Age 2-24 months Immunosuppression, CDC stage A, B, C Prior anitretroviral treatment <6 wekks	8	24	38% ↓1.5 $\log_{10}$ cop/ml 25% <400 cop/ml	Stable of slight increase (88% was not immunosuppressed)	NA	(6)
Hainaut et al.	1. ZDV+ddI+ 3TC 2. ZDV+3TC +NVP	120 mg/m^2/day first 14 days followed by 300-400 mg/m^2/day	Children born to HIV-1 infected mothers that became HIV-1 positive during perinatal follow-up	4	NA	50% <50 cop/ml	Persistent normal CD4+ T-cell counts	No side-effects	[(41)

Table 6: Pediatric studies using Efavirenz (EFV)

Author	Regimen	EFV Dose	Inclusion criteria	Number of patients	Follow - up (weeks)	Outcome HIV-1 RNA	Outcome CD4+ T-cells	Toxicity	Reference
Teglas et all	1. EFV + NRTI (n= 24) 2. EFV +PI +NRTI (n=9)	Median dose 13.3 mg/kg	inclusion criteria not included in paper	33	24 weeks	Med ↓1.2 $\log_{10}$ cop/ml 48 % below 200 copies/ml 27 % below 50% copies/ml	Med ↑ 128 cells/mm^3	42% of the children had at least one distinguishable side effect. Of all children 15% suffered of cutaneous side effects, 30% of nervous system side effects and 6% of both. 7 children stopped the study due to intolerance	(43)
Starr et al.	NFV+EFV+ 2NRTIs	Mean dose at 2 weeks 11.7 mg/kg Mean dose at 6 weeks 13.3 mg/kg	Age < 16 years, HIV-1 RNA >400 cop/ml, PI and NNRTI naive, Ability to swallow capsules	48	57	Med ↓ 2.7 $\log_{10}$ cop/ml 81% <400 cop/ml (AT) 61% <400 cop/ml (ITT) 70% <50 cop/ml (AT) 53% <50 cop/ml (ITT)	Med ↑ 74 cells/mm^3 =3% of total T-cells	25% moderate, 9% severe , 2% life threatening. Most common:rash, diarhhea, neutropenia, biochemical abnormaillities	(21)

Table 7: Pediatric study using abacavir (ABC)

Author	Regimen	ABC Dose	Inclusion criteria	Number of patients	Follow - up (weeks)	Outcome HIV-1 RNA	Outcome CD4+ T-cells	Toxicity	Reference
Sáez-Llorens et al.	1. ZDV+3TC (n=103) 2. ABC+ZDV +3TC (n=102)	8 mg/kg q12h	Age 3 months-13 years, CDC stage N,A,B,C, PI discontinuation ≥2 weeks before enrollment, Normal hematology and chemistry parameters, CD4+ % >15%	205	48	1. Med ↓0.21 log_{10} cop/ml 1. 6% <400 cop/ml 2. Med ↓0.61 log_{10} cop/ml 2. 11% <400 cop/ml	1: Med ↑-14 cells/mm^3 =0.8% of total T-cells 2: Med ↑99 cells/mm^3 =3.1% of total T-cells	Nausea, vomiting and cough more frequent in 2 (46% and 46^ versus 30% and 25% resp.) Fever, diarrhea, nasal signs, rashes and ear/nose/throat infections occurred in ≥15% Grade 3 or 4 infrequently	(45)

Administration of NFV plus a NNRTI (nevirapine or efavirenz) and two NRTI's is associated with excellent virological responses. (21, 33, 34)

Wiznia et al compared the effectiveness of NFV, NVP, d4T and 3TC to NFV, d4T and 3 TC. The virological and immunological responses were superior with the four-drug regimen. (21, 33, 34) Moderate to severe (≥ grade 2) side-effects were frequently observed in 25% to 77% of the patients. Adverse events included diarrhea, nausea, vomiting and rash. A transient or persistent increase in the serum levels of triglycerides or cholesterol was observed in 50% and 30% respectively, although no clinical signs of lipodystrophy were reported.

Schuster et al. and Wiznia et al. reported that the administration of NFV in a twice daily regimen results in pharmacokinetic parameters which are comparable to those found in a three times daily regimen. (33, 34) However, pediatric studies on the efficacy of a twice daily regimen are not available. In Table 3 the pediatric studies with NFV are summarized. Nelfinavir is the only protease inhibitor with a pediatric formulation (powder) that is easy to use in very young infants as well as in older children. However, the co-administration with food may result in problems in children that refuse to eat or in children that have to be awakened for an evening dose. Again, the study results with NFV differ widely. In one study a virological response rate was demonstrated comparable to that obtained in adults. Combination treatment of NFV with EFV resulted in an excellent virological response rate. However, when children fail on this regimen, few alternatives are left, because of cross-resistance within these groups of antiretroviral medication. Gastro-intestinal side-effects are frequently associated with the use of NFV, but are mainly transient. Increases in triglycerides and cholesterol have been reported in 31 to 91% of the children which may have important consequences for their health. The efficacy of a twice-daily regimen, which seems possible from a pharmacokinetic point of view, should be studied.

Saquinavir

Saquinavir hard gelatin capsule (SQV-HGC) was the first HIV-1 protease inhibitor approved for use in HIV-1-infected adults by the FDA. The use of SQV-HGC has been restricted by poor bioavailability (4% uptake of a single oral dose taken with food). To improve this pharmacokinetic profile SQV has been combined with RTV or with NFV. Both PI's are inhibitors of cytochrome P450 3A isoenzymes and therefore co-administration results in markedly higher plasma concentrations of SQV.

Hoffmann et al. studied the efficacy of SQV with RTV and of SQV with NFV in 11 children with failure on prior therapy including at least one PI. (35) The antiretroviral effect (HIV-1 RNA reduction and CD4+ T-cell increase) was more pronounced with the combination SQV/RTV than when SQV/NFV were given. This was probably due to the stronger inhibition of cytochrome p459 3A isoenzymes by ritonavir thus leading to higher trough levels of SQV in combination with RTV. Complete suppression of viral replication below quantifiable levels could not be achieved. Both regimens were well tolerated. Recently a new formulation of SQV that improves the low oral bioavailability of SQV-HGC, saquinavir soft gelatin capsule (SQV-SGC) has been approved for use in HIV-1 infected adults. Kline et al. evaluated the pharmacokinetics, tolerance, safety and activity of SQV-SGC with NFV in children older than 3 years and reported good tolerance, safety, and virological and immunological response rates. (36) In Table 4 the results of pediatric studies with SQV are

summarized. The combination of SQV-SGC with NFV results in a very promising virological suppression in the absence of serious side-effects.

Nevirapine

Nevirapine (NVP) is a potent non-nucleoside inhibitor of HIV-1 reverse transcriptase with a favourable pharmacokinetic profile allowing twice daily administration. (37, 38) In adults once daily dosing results in similar exposure to NVP. *(39)* However, pharmacokinetic evaluation has not yet been performed in children. NVP is available in a pediatric formulation (liquid).

Luzuriaga et al. studied the pharmacokinetics, safety and activity in HIV-1 infected children in 1996. (40) Administration of NVP leads to a rapid and profound (≤ 50% of baseline) reduction in plasma p24 antigen levels in 10 of 12 children. However, at the lower dosage (120 mg/m^2/day) antiviral activity was lost rapidly and appeared to be associated with the isolation of viruses with decreased in vitro sensitivity. At higher dosages a more durable antiretroviral activity was observed during the 8 weeks of monotherapy. Rash was the most common side-effect in 5% of the patients. Because the use of NVP in combination with other antiretroviral agents would likely provide more potent antiretroviral activity, Luzuriaga et al. studied combination treatment with NVP, zidovudine and lamivudine in 8 infants younger than 16 months. In this study a more durable response was found. (6) However, only 25% of the patients reached an HIV-1 RNA load <400 copies/ml. Viruses with decreased sensitivity to nevirapine were isolated during the treatment period from five infants. (6) Hainaut et al. treated 2 vertically infected infants with NVP, ZDV and 3TC. In both infants an HIV-1 RNA load <50 copies was observed. (41)

Table 5 summarizes the pediatric studies with NVP. The development of class resistance with single step mutations in the reverse transcriptase gene remains a major therapeutic problem with this class of antiretrovirals. Since HIV-1 infected children tend to have higher baseline viral loads with a higher replication rate than adults, this is a major disadvantage in the treatment of children with a high baseline viral load. *(42)* However, the favourable pharmacokinetic profile allowing twice and possibly once daily dosing, a pediatric formulation with a taste that children like and the absence of major side-effects makes this drug a promising antiretroviral component for children with lower baseline viral loads.

Efavirenz

Efavirenz (EFV) was the second NNRTI to be approved for the use in children. A pediatric (liquid) formulation is currently available on a compassionate use basis. The pharmacokinetic profile of EFV allows for once daily dosing. In spite of this promising feature, little experience has been obtained with the treatment of HIV-1 infected children with EFV. Star et all. conducted a study in children receiving EFV in combination with NFV and NRTI. *(21)* A total of 57 HIV-1-infected children were included. After 48 weeks of treatment 76% of the children had plasma levels below 400 copies and 63% below 50 copies. High viral loads at start of the study decreased the likelihood that plasma viral loads would become undetectable. Adverse effects of at least moderate severity included rash (30%), diarrhea (18%), neutropenia (12%) and biochemical abnormalities (12%). Mild central nervous system toxicity was found in 14% children, which resolved once EFV was given at bedtime

rather than in the morning. Since the patients in this study received a combination of NFV and EFV it is impossible to discriminate which of both drugs induced this toxicity. Teglas et al. conducted a study in 24 children receiving EFV and a NRTI and 9 children receiving EVF, a NRTI and a PI. *(43)* After 6 months of treatment the viral load was below 200 copies in 48% of the children and below 50 copies in 27% of the children. Fifteen children (42%) suffered of at least one clinically apparent side effect. Five (15%) had mild diffuse cutaneous eruptions, 10 (30%) children suffered from nervous system side effects and two had both. The treatment was discontinued due to intolerance in seven children. Younger children experienced more side effects than older children. Table 6 summarizes the results of studies with EFV.

In children the use of efavirenz has been associated with serious adverse events. Rash and serious neurological problems are frequent side-effects. High viral loads at start of the study decreased the likelihood that plasma viral loads would become undetectable. Therefore, in our opinion efavirenz is not a first choice drug in children.

Abacavir

Abacavir (ABC) is a potent nucleoside reverse transcriptase inhibitor (NRTI) that in combination with two other NRTI's results in a viral load of <400 copies/ml in 74% of treatment-naive adults after 48 weeks of therapy. (44) It is available in a pediatric formulation (liquid). Sáez-Llorenz et al performed a randomized, double-blind study of ABC/ZDV/3TC versus ZDV/3TC in 205 antiretroviral therapy-experienced HIV-1 infected children. (45) Virological, immunological and clinical response rates over the 48 weeks of the study indicate that the addition of ABC to ZDV/3TC provided increased antiviral activity over that provided by ZDV/3TC. However, as expected in antiretroviral therapy-experienced participants many of whom had received previous therapy with ZDV with of without 3TC, the degree of viral suppression provided by the ABC/ZDV/3TC regimen was modest, while improvement in immune response was moderate. Nausea/vomiting and cough occurred more frequently among children receiving ABC/ZDV/3TC. The hypersensitivity syndrome associated with the use of ABC was not observed in this study. Thus far only one child (3%) with a hypersensitivity reaction has been reported in a phase I study of ABC in HIV-1 infected children. (46) In Table 7 the results of the pediatric studies with ABC are summarized. The well tolerated pediatric formulation, the possibility to administrate this medication twice-daily and the low incidence of serious side-effects result in favourable qualities of ABC. However, studies on the efficacy of this drug in naive children are lacking. Since in adults poorer virological outcome has been associated with a high baseline viral load and children tend to have higher baseline viral loads, the efficacy of ABC in children should be studied. (42, 47)

Discussion

This review provides an overview of all current articles on pediatric clinical trials using HAART. Twenty-three papers mostly with a small number of patients were available using 4 PI's (RTV, IDV, NFV and SQV), 1 NNRTI (NVP) and 1 NRTI (ABC). HIV-1 RNA loads and CD4+ T-cell counts were used as primary outcome measures, because these parameters have been demonstrated to be independent predictors of the clinical course in HIV-1 infected infants and children. (3) In addition,

these two surrogate measures were measured in all studies, while other clinical progression markers such as growth (48) were unfortunately often not available.

Virological response rates were highly variable both between the different antiretroviral drugs but also between different studies using the same medication. The studies with the highest percentages of children reaching a viral load below the detection limits of 400 copies/ml were reported by Starr et al: NFV+EFV+2 NRTI's, Vigano et al.: IDV+d4T+3TC, Kline et al.: SQV+NFV+2 NRTI's, Van Rossum et al.: IDV+ZDV+3TC, Funk et al.: NFV+2NRTI's and Wiznia et al.: NFV+NVP+2 NRTI's. (20, 21, 23, 24, 33, 36) The percentages of viral loads below the detection limit of 400 copies/ml varied between 63% and 87%, which is comparable with the data on HAART in adults. (25, 26) However, the majority of pediatric studies showed inferior virological response rates in comparison with those in adults. In studies using RTV, NFV, IDV, NVP or ABC virological success rates varied from 11% to 50% of the patients with a HIV-1 RNA load <400 copies/ml. (4-11, 22, 27-29, 33, 45)

Several factors may be associated with virological failure. These include pharmacokinetic parameters (low plasma blood levels of protease inhibitors are associated with virological failure (49, 50)), inadequate adherence to antiretroviral therapy and differences in baseline characteristics (prior antiretroviral treatment, younger age, and high baseline viral load). Since studies were often not comparable with respect to these factors, comparison of the results of the different studies is complicated. It is striking that all four studies in which dosages of the administrated PI were adjusted after pharmacokinetic evaluation resulted in superior virological response rates compared to studies in which fixed dosages were used. (21, 23, 24, 36) Interindividual pharmacokinetic differences result in inadequate plasma PI levels in a part of the children treated with a fixed dose. (27, 34, 51-53) Since virological response is associated with plasma protease inhibitor levels, these inadequate plasma PI levels may be partly responsible for the differences in virological response rates. (27, 28, 32, 51, 52) In our opinion, it is therefore imperative to measure PI pharmacokinetics in all children treated with a PI to determine the individual dosage necessary for pharmacokinetic values comparable with adult values.

The combination of nelfinavir with either a NNRTI or SQV and two NRTI's resulted in optimal virological responses in a high percentage of the patients. These twice daily regimens may result in an improved adherence to HAART compared to three daily medication schemes. (21, 33, 36) Lopinavir/ritonavir, which was recently approved by the FDA is also a very promising compound because of twice daily dosing and the excellent virological response rates. (54) Future possibilities for once daily -or even less frequent- dosing are very important to improve adherence to antiretroviral therapy. Administration of indinavir with two NRTI's also leads to a good virological response rate in a high percentage of the patients. (20, 24) However, in a majority of the studies with indinavir virological response rates were suboptimal. (4, 5, 7-9, 28, 29) Since these studies differed substantially in follow-up time, baseline characteristics of the patients and study design these variable results are not well understood.

Analyses of T-cell repopulation in groups of children with different ages are hampered by the fact that CD4+ T-cell counts are highly dependent on the age of the patients. (55, 56) The calculation of CD4+ T-cells as a percentage of the total T-cell counts is therefore used in many pediatric studies. However, data generated in this way are influenced by the major changes in the numbers of CD8+ T-cells in HIV-1-infected patients. (57-59) Immunological outcome parameters of

the different pediatric studies are therefore not comparable. Nevertheless, immunological response rates were more uniform than the virological responses. In almost all studies increases of CD4+ T-cell counts are reported independent of the extent of the virological response. CD4+ T-cell numbers in HIV-1-infected children on HAART recover more rapidly than CD4+ T-cells in HIV-1-infected adults. (12, 57, 60-63) The good immunological response to HAART has been attributed to the large volume of the thymus, which is still present in young children. (64, 65) The observation that CD4+ T-cell counts recover despite the presence of virological failure may possible be explained by the selection of certain viral variants with resistance to protease inhibitors that have in-vitro impaired replicative capacity. (66) Douek et al. also reported an increase in peripheral CD4+ T-cell counts in both virologic responders and non-responders on antiretroviral therapy. (67) However, they observed that the recovery of thymic function was affected by the degree to which virus suppression was achieved when thymic function was measured by quantifying T-cell receptor rearrangement excision circles in peripheral blood.

Side-effects of HAART were generally mild, transient and mainly of gastro-intestinal origin. Lipodystrophy syndrome is commonly reported in HIV-1 infected adults treated with antiretroviral therapy. The occurrence of this syndrome consisting of a fat-wasting condition associated with abnormalities of lipid metabolism and impaired glucose tolerance has been reported in 1.5-83% of HIV-1-infected adults. (68, 69) Only two groups investigated the incidence of lipodystrophy in children. The incidences in these studies were 29% and 33%. (70, 71) In both studies lipodystrophy was associated with advanced disease at baseline. Jaquet et al. observed a combination of peripheral atrophy and central adipositas in only two of a group of 39 pubertal children. In the other children only one of these features was observed. (71) Significant percentages of patients with serum lipid abnormalities were reported in three pediatric studies. (11, 23, 35) However, the investigators did not report signs of clinical lipodystrophy. The consequences of the lipodystrophy syndrome for the future treatment of HIV-1 infected children are not yet clear, because little is known about the pathophysiology, although it is generally believed that lipodystrophy has multiple causes and modes of presentation. Furthermore, it still remains to be clarified whether this syndrome is attributable to antiretroviral drugs. Another concern in long-term treatment of HIV-1 infected children is the observation of an increased rate of bone turnover causing bone mineral density decrease. The severity of osteopenia seems to be related to lipodystrophy. *(72)* Long-term data on the comparison between PI containing regimens versus NNRTI containing medication schemes with respect to lipodystrophy and bone mineral loss are necessary to evaluate the contribution of the different antiretroviral drugs to the development of these abnormalities.

The inferior virological response rates, which have been reported in HIV-1 infected children treated with HAART form a reflection of the challenges which are encountered in the treatment of children with HIV-1/AIDS. Difficulties with adherence and with the pharmacokinetics of protease inhibitors in children demand an intensive, child adjusted approach. Since HIV-1 infection in children is a rare disease in the Western world, and experience in the treatment is often insufficient, it may be more practical to treat children with an adjusted regimen that is not dependent of pharmacokinetic evaluation. This could be achieved by initiating therapy with a potent regimen to reduce the viral load to undetectable levels. Lopinavir/ritonavir is a very potent protease inhibitor and could thus be part of this induction regimen. Since drug levels are about 40 times above the IC50, pharmacokinetic monitoring is not necessary. However, long-term treatment with

lopinavir/ritonavir in children is complicated by the poor taste of lopinavir/ritonavir and by serious changes of lipid metabolism. (54) After viral load has been significantly reduced, an easy to use maintenance regimen with less side-effects could be introduced to maintain long-term adherence. This maintenance regimen may contain for example nevirapine or abacavir. Future studies with induction-maintenance regimens are needed. The goal of these studies is to ensure similar efficacy and toxicity compared to those reported in adults.

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Clinical and virologic response to combination treatment with indinavir, zidovudine and lamivudine in HIV-1 infected children: A multicenter study in The Netherlands

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Chapter 9

Abstract

Objective: To evaluate the clinical, immunologic and virologic response to indinavir, zidovudine and lamivudine in children with HIV-1 infection. *Study design:* Twenty-eight HIV-1 infected children, 3 months to 16 years of age with or without prior treatment with reverse transcriptase inhibitors and a viral load of more than 5000 copies/ml and/or a CD4 cell count less than the lower limit of the age-specific reference value were treated with indinavir, zidovudine and lamivudine. Pharmacokinetics of indinavir were determined in each child. *Results:* The combination treatment was well tolerated in the majority of the patients. In three patients, a transient hematuria was seen. Clinical improvement was seen in all patients. The median baseline HIV-1 RNA was 5.1 $\log_{10}$ copies/ml. After six months of therapy, 70% of the patients had a HIV-1 RNA load below the detection limit of 500 copies/ml, whereas 48% of the children had a viral load below 40 copies/ml. Relative CD4 cell counts in relation to the lower limit of the age-specific reference value increased significantly from a median value of 79% at baseline to 106% after six months of therapy. The doses of indinavir necessary to achieve AUC-values comparable to adult values varied from 1250 mg/m^2/day to 2450 mg/m^2/day. *Conclusions:* HAART consisting of indinavir, zidovudine and lamivudine in children reduced HIV-1 RNA to less than 500 copies/ml in 70% of the children within six months. In most of the patients improved CD4 cell counts were observed, as was a better clinical condition (no invasive or opportunistic infections, increased weight gain). Side effects of the triple therapy were mild and mainly of gastro-intestinal origin. HAART may therefore be used as successfully in children as in adults.

Introduction

Since its introduction in 1996, combination therapies which include two reverse transcriptase inhibitors and a protease inhibitor have rapidly become standard therapy for HIV-1 infected adults.(1) However, so far the data on efficacy of these combinations in children with HIV-1 infection are limited, and are derived from studies with only small numbers of children. The relative immaturity of the immune system, differences in pharmacokinetics and pharmacodynamics of antiviral drugs and specific issues concerning adherence to therapy, complicate the extrapolation from therapeutic results in adults to those in children.

Recent studies in children with HIV infection treated with a combination of two reverse transcriptase inhibitors showed superior results as compared to monotherapy.(2-12)
Alternatively, monotherapy with the protease inhibitor indinavir resulted in marked –but only temporary- reductions of viral load in antiretroviral therapy naïve and experienced adult patients, as well as in children. (13, 14)

Several small observational and retrospective studies reported that triple therapy including a protease inhibitor may be as effective in pre-treated HIV-1 infected children as in adults, as shown by a decrease of viral load and an increase of CD4 cell count similar to those in antiretrovirally experienced adults. (4, 15-20) The measurement of these two surrogate markers has largely become the basis for the prediction of clinical and virologic response in adults. (21) Plasma HIV-1 RNA levels and CD4 lymphocyte counts are also independent predictors of the clinical course in HIV infected infants and children.(6)

Here we report the results of the initial six months in a prospective, open, uncontrolled, Dutch multicenter study, which evaluates the clinical, immunologic and virologic response to combination therapy with indinavir, zidovudine, and lamivudine in children with HIV-1 infection.

Methods

Inclusion and exclusion criteria

Children between the ages of 3 months and 18 years were eligible for enrolment. Children were included when the plasma HIV-1 RNA test was positive on two subsequent occasions (children less than 18 months old) or when the HIV serology was positive (children more than 18 months old) in the presence of one of the following abnormal test results: a mean HIV-1 viral load of more than 5000 copies/ml (mean of two measurements with less than four weeks in between) and/or a CD4 cell counts below 1 year: < 1750/mm^3, 1-2 years: <1000/mm^3, 3-6 years: <750/mm^3, >6 years: <500/mm^3 (22). Patients were excluded when they had been treated with antiviral agents other than zidovudine and/or didanosine or zalcitabine. There were no restrictions with regard to gender, ethnicity, or route of acquiring HIV-1 infection.

Study protocol

The study protocol was approved by the medical ethical committees of all the participating centers. Written informed consent was obtained from parents or legal guardians. Blood samples were taken twice before triple therapy was started (4-2 weeks and 2-0 weeks prior to initiation of the study) for hematological parameters (hemoglobin, hematocrit, white blood cell count, differential blood cell count, platelet count), serum biochemistry (sodium, potassium, calcium, albumin, liver enzymes, creatinine, urea, amylase, total bilirubin), immunoglobulin analysis, including IgA, IgG and IgM serum levels, virologic and immunologic parameters. The same tests were performed at days 7, 14 and 28 after initiation of triple therapy and after 2, 3 and 6 months. Urine was collected for analysis at all timepoints. At each visit a physical examination was performed, weight and length were measured and parents were asked for the presence of adverse events. Length-for-age scores and weight-for-length scores were determined using the Dutch reference curves for age and gender. (23) These scores were also available for twenty-one children in the lifespan before HAART was started (hospital medical records and/or the school doctor or health center). We defined response of growth to treatment as catch-up up growth to the original percentile or showing an end to the ongoing deviation. in children with an impaired growth (deviation from the original percentile of length-for-age score and/or weight-for-length score) at baseline. Lymphocyte subsets were analyzed with the FACSCount System (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA).(24) Plasma HIV-1 RNA quantitation was analyzed by an *in vitro* (-PCR) test (most samples: Amplicor HIV-1 monitor test version 1.5 Roche Diagnostic Systems and a few samples: NASBA assay, nuclisensHIV-1 RNA; Organon Teknika Boxtel, The Netherlands) with a lower limit of detection of 500 and 400 copies/ml. If plasma HIV-1 RNA was below this lower limit, plasma samples were subsequently tested with a modified, ultrasensitive procedure that increases the analytical sensitivity of the Amplicor HIV-1 monitor test to a detection limit of 40 copies/ml.(25)

Medication and pharmacokinetics

Medication was given in the following doses: indinavir 400 mg/m^2 every 8 hours, zidovudine 120 mg/m^2 every 8 hours and lamivudine 4 mg/kg every 12 hours. Indinavir was administered as standard-capsules of 200 mg and 400 mg or as capsules of 150 mg and 300 mg, which were prepared at the pharmacy. When patients were too young to swallow capsules, indinavir capsules had to be opened by the caregivers and dissolved in five to ten milliliters of water. In children in whom problems were encountered with the ingestion of indinavir, the drug was changed into nelfinavir (30 mg/kg every 8 hours). Zidovudine was administered either as syrup (10 mg/ml) or as capsules of 100 mg and 250 mg depending on the child's age. Lamivudine was administered as syrup (10 mg/ml) or as tablets of 150 mg. The importance of taking the indinavir one hour before or two hours after meals was repeatedly emphasized. Low fat food and drinks were allowed in this period before and after the medication. Parents received a list with low fat food and drinks that they could give to their children in this period. A day to day medication scheme including the times when no fat food or drinks were allowed was also given.

At day 28 patients, were admitted to the day-care unit to determine the steady state pharmacokinetics of indinavir. When a dosage adjustment of indinavir was necessary to normalize the area under the curve-concentration (AUC) curve to adult values (20 mg/L*hr, range 10-30 mg/L*hr), this procedure was repeated.

Compliance

Drug- compliance was assessed by interviews of the parents, by measurement of indinavir plasma levels and by medication diaries, which were made for each child individually. When possible children who were old enough were asked to apply stickers in their diaries every time they took their medication. These diaries were checked at every visit. When there were problems with compliance, coaching of parents and children was intensified.

In the analyses poor compliance was defined as the patients in whom the interviews of the parents, the medication diaries and/or plasma levels of indinavir showed that serious (i.e. more than one time) problems with adherence to the medication scheme existed. Patients with good compliance were defined as the patients in whom according to the interviews, the medication diaries and the plasma levels of indinavir no problems were found.

Adverse Events

Adverse events were defined as any clinical sign or symptom, or meaningful laboratory-test abnormality, excluding disorders associated with HIV-1 infection. All adverse events were rated according to severity and relation to study drug.

Statistical Analyses

The primary measures of antiretroviral-drug activity were the magnitude and duration of changes in plasma HIV-1 RNA and CD4 cell counts over a period of 6 months. One child dropped out of the study within one week (lost to follow-up). As no follow-up data of this child were available, the remaining twenty-seven patients were included in the intention to treat analyses. In the determinations of the median change from baseline, viral loads of patients below the detection limit were considered to be 40 copies/ml. For occasional missing data in this intention to treat analysis,

the last observation carried forward method was used to obtain viral loads. In 5.4% of the patients one or more values at key analysis time points (median: 1, range 1 to 4) were missing. Relative CD4 cell counts in relation to the lower limit of the age-specific reference value (22) were calculated by dividing the individual value at the different time points by the lower limit of the reference value. Changes of CD4 cell counts and viral load results were evaluated using the Wilcoxon signed rank test. The relation between the plasma HIV-1 RNA levels at month six and various characteristics (baseline viral load, prior treatment, compliance) was investigated using multiple linear regression analysis for normally distributed data. In this analysis both viral load results were logarithmically transformed, and allowed for the fact that various children at month six had an HIV-1 RNA load which was actually lower than the detection limit (i.e. left-censored data). (26) All p-values are two-tailed.

Results

Twenty-eight HIV-1 infected children were enrolled at 5 centers between April 1997 and July 1998. The evaluation of the first 24 weeks is presented in this report. Base line characteristics of the twenty-seven children of whom follow-up data are available are presented in Table 1. The median age of the children was 6.0 years (range: 3 months –16.6 years), twelve patients were not previously treated and fifteen had received prior treatment with nucleoside reverse transcriptase inhibitors, mostly with zidovudine monotherapy for an average of 34 months (range: 8-118). The median HIV-1 RNA level was 127,500 copies/ml (range: 725-761,500). Eight children were too young to swallow capsules and received indinavir dissolved in water. Despite its bitter taste, six of these young children had no problems with it. Two other infants had problems with the administration of indinavir necessitating a change of indinavir into nelfinavir within two weeks after start of the therapy.

In 19 of 27 patients (70%) the dose of the indinavir had to be increased according to the pharmacokinetics to achieve AUC-values between 10 and 30 mg/L*hr. In nine patients the dose had to be increased to 1500 mg/m^2/day, in six patients to 1800 mg/m^2/day, in one patient to 2000 mg/m^2/day and in two patients to 2450 mg/m^2/day. In seven patients 1250 mg/m^2/day was adequate. Because two patients were administered nelfinavir at day 28, no steady state pharmacokinetics of indinavir were determined in these patients. The pharmacokinetics of indinavir will be presented in more detail elsewhere.

Clinical results

All patients improved in their overall condition as shown by increased activity, an increased appetite and well being, as reported by their caregivers. No serious invasive or opportunistic infections were seen. In 81% (17/21) of the children a deviation of the original percentile in the length-for-age and/or weight-for-length reference curve (5 children a deviation in the length-for-age curve, 2 children a deviation in the weight-for-length curve and 10 children in both curves) was observed at baseline. A response (catch-up growth or the end of ongoing deviation) was seen in 76% (13/17) of these patients. None of the patients showed progression of their CDC- classification stage and no AIDS-defining events did occur. Two patients were admitted to the hospital for a short period (ten

days): one because of hematuria and vomiting and the other because of hematuria and hypertension.

Table 1: Baseline characteristics of the study patients

Baseline characteristics								
Median age in years (range)	6.0 (0.3-16.6)							
Sex, male/female	14/13							
Race/ethnicity:								
Non-white	23							
White	4							
Route of acquisition:								
Vertical	21							
Blood products	4							
Unknown	2							
Clinical stage (CDC-classification)*:	N1	2	A1	3	B1	0	C1	0
	N2	3	A2	4	B2	5	C2	1
	N3	0	A3	4	B3	1	C3	4
No prior treatment:	14							
Prior treatment with:								
Zidovudine	11							
Zidovudine/zalcitabine	2							
Median copies of HIV RNA/ml plasma (range)	127,500 (725-761,500)							
Naive to antiretroviral therapy	210,000 (23,900-761,500)							
Experienced to antiretroviral therapy	23,250 (725-718,00)							

*Clinical and Immunologic categories as defined by the US Centers for Disease Control and prevention (CDC). (32)

HIV-1 virologic responses

The levels of the viral load after start of HAART are demonstrated in Figure 1. Figure 1A shows the levels of HIV-1 RNA of the patients with a good compliance. Figure 1B shows the levels of the viral load from the eight children with a poor compliance and from the two children who switched to a different medication scheme within two weeks after the treatment was started. One of the latter two children had also a poor compliance. Table 2 shows the median changes from baseline of the viral load and the proportion of patients whose viral load levels decreased to less than 500 and less than 40 copies/ml in an intention to treat analysis.

In Figure 2, scatterplots of the changes in viral load from baseline to month 6 against the baseline viral load are shown. Figure 2A shows the change of HIV-1 RNA from baseline to month 6 in all patients. Although the number of patients with poor compliance is small, these patients have less change in HIV-1 RNA load compared to the patients with good compliance. In almost all patients with good compliance throughout the range of baseline HIV-1 RNA loads an optimal response is seen. The two patients with the highest viral loads had an almost optimal response 6 months after the start of combination therapy. In Figures 2B and 2C the patients with prior treatment are separated from the naïve patients. In all pre-treated patients with a good compliance an optimal response was seen and in all pre-treated patients with poor compliance a suboptimal response was seen. However, three of the five patients with prior treatment and with a poor compliance had a high baseline viral load (median 260,000, range 725-718,000 copies/ml), whereas all pre-treated

patients with a good compliance had a relatively low viral load (median 43,985, range 2,680-761,500). Using multiple regression analysis the plasma HIV-1 RNA at month six did not significantly correlate with baseline viral load and prior treatment. In case of poor compliance the median HIV-1 RNA load at month six was found to be 158-fold higher ($p<0.001$) as compared to patients with an adequate compliance.

The reduction in log HIV-1 RNA was found to be different between children whose initial dose appeared adequate compared with those whose AUC values required an increase in dose. Baseline HIV-1 RNA and the initial response until two months after the start of the therapy did not differ between the two groups. However, three and six months after the start of the therapy a statistically significant difference ($p= 0.026$ and $p= 0.014$) was found.

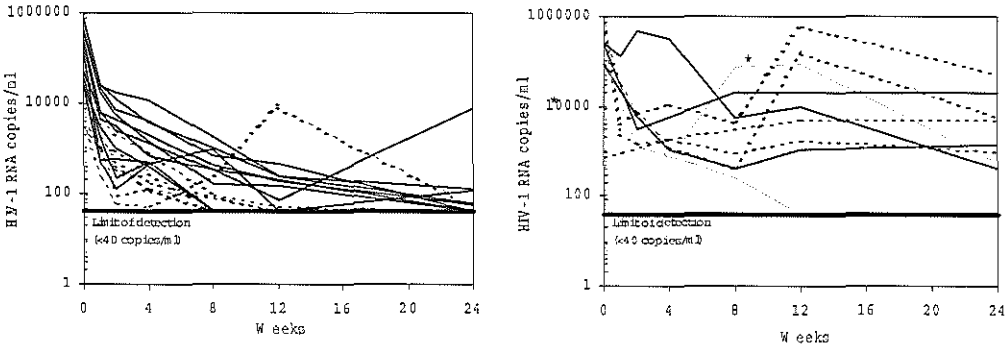

Figure 1A: *Levels of HIV-1 RNA in 18 patients who completed therapy without documented poor compliance. Note the non-linearity of the horizontal axis. Patients who were experienced to antiretroviral treatment are represented by the dotted curves.* ***Figure 1B:*** *Levels of HIV-1 RNA of the seven patients with documented poor compliance and the two patients (grey curves) who switched to a different medication scheme within two weeks after the start of the therapy. One of the latter was also non-adherent (indicated by *).*
Patients who were experienced to antiretroviral treatment are represented by the dotted curves.

CD4 cell counts

Because absolute CD4 cell counts are highly dependent on the age of the patients and on the stage of disease of the patients, relative CD4 cell counts in relation to the lower limit of the age-specific reference value were calculated. Figure 3A shows relative CD4 cell counts in patients of all age groups. In the whole group of twenty-seven patients the median relative CD4 cell count (range) was 79 % (2-250) at the start of the therapy and 69% (2-208) after 1 month of therapy. After 3 months there was an increase to a median of 82% (4-278) and after 6 months a significant ($p= 0.007$) increase to a median of 106% (2-266) was seen. Figure 3B shows the absolute CD4 cell counts in three different age groups (<2 years, 2-5 years and > 5 years). The median absolute CD4 cell count (range) of all patients was 0.61 (0.01-4.99) at baseline, 0.52 (0.01-3.19) after 1 month, 0.66 (0.02-3.56) after 3 months and 0.71 (0.01-4.38) after 6 months of treatment ($p= 0.04$).

If patients with documented poor compliance were excluded, increases of CD4 cell counts were also not significant at time points before six months of therapy; after one month the median relative CD4 cell count decreased from 74% (2-250) before start of the therapy to 68% (2-208).

After three months relative CD4 cell counts were 76% (14-278) and after six months 106% (40-266) (p= 0,009). Figure 3C shows the absolute CD4 cell counts in three different age groups (<2 years, 2-5 years and > 5 years). The median absolute CD4 cell count (range) in these patients was 0.61 (0.01-4.99) at baseline, 0.51 (0.01-3.19) after 1 month, 0.64 (0.07-3.56) after 3 months and 0.78 (0.2-4.38) after 6 months of treatment (p= 0.05). In patients with documented poor compliance no significant changes of the CD4 cell counts were seen.

Table 2: Virologic results

Weeks after start of the treatment	**Median change of viral load from baseline (range) All p <0.001**	**Viral load < 500 c/ml**	**Viral load <40 c/ml**	**Viral load <500 c/ml in case of good compliance**	**Viral load <40 c/ml in case of good compliance**
1	**Tot: -1.26 (-2.45 to +0.70)** Na: -1.29 (-2.45 to +0.70) Exp: -1.24 (-2.25 to 0.00)	0%	0%	0%	0%
2	**Tot: -1.69 (-3.05 to +0.26)** Na: -1.76 (-3.05 to +0.26) Exp: -1.59 (-2.86 to 0.00)	0%	0%	0%	0%
4	**Tot: -1.91 (-2.50 to +0.43)** Na: -1.98 (-2.50 to +0.08) Exp: -1.90 (-2.44 to +0.43)	**Tot: 33% (9/27)** Na: 20% (3/15) Exp: 50% (6/12)	0%	**Tot: 47% (9/19)** Na: 25% (3/12) Exp: 86 % (6/7)	0%
12	**Tot: -2.63 (-3.56 to +0.85)** Na: -2.96 (-3.56 to -0.35) Exp: -1.94 (-3.13 to +0.85)	**Tot: 67% (18/27)** Na: 80% (12/15) Exp: 50% (6/12)	**Tot: 37% (10/27)** Na: 33% (5/15) Exp: 42% (5/12)	**Tot: 95% (18/19)** Na: 100% (12/12) Exp: 86% (6/7)	**Tot: 53% (10/19)** Na: 42% (5/12) Exp: 71% (5/7)
24	**Tot: -2.78 (-4.09 to +0.85)** Na: -2.96 (-4.09 to -1.10) Exp: -1.94 (-3.24 to +0.85)	**Tot: 70% (19/27)** Na: 80% (12/15) Exp: 58% (7/12)	**Tot: 48% (13/27)** Na: 47% (7/15) Exp: 50% (6/12)	**Tot: 95% (18/19)** Na: 92% (11/12) Exp: 100% (7/7)	**Tot: 68% (13/19)** Na: 58% (7/12) Exp: 86% (6/7)

Tot= total of the patients, Na= naive to antiretroviral therapy at baseline, Exp= experienced to antiretroviral therapy at baseline

Adverse Events

Combination therapy was well tolerated by almost all children. In two children, medication had to be switched to another combination within two weeks after starting indinavir. This was due to vomiting in one child and because of extreme dislike of the medication in another infant that could not swallow capsules. Five other patients had complaints of vomiting, three of nausea, two had diarrhea and four patients had a skin rash. Three patients had loss of appetite, three patients loss of weight and 3 patients hematuria, which resolved after discontinuing medication for two days and re-emphasizing the importance of adequate fluid intake. In total eleven patients (41%) of the twenty-seven patients studied had adverse events.

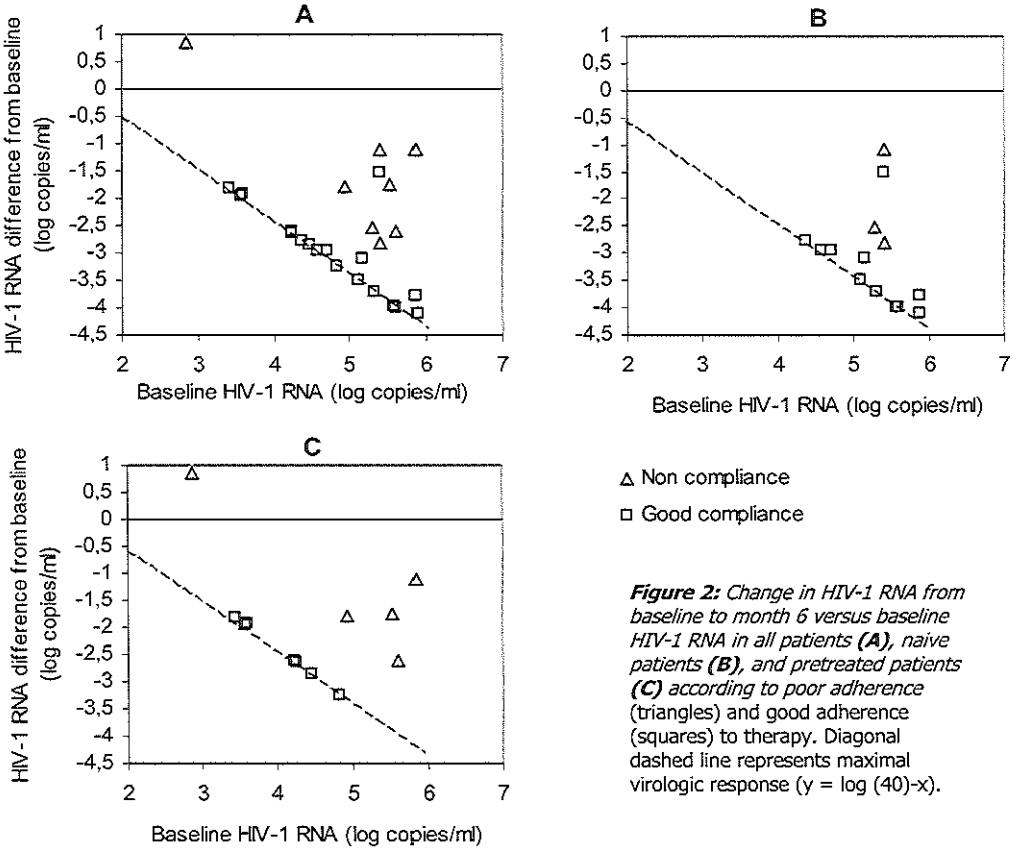

Figure 2: *Change in HIV-1 RNA from baseline to month 6 versus baseline HIV-1 RNA in all patients* ***(A)****, naive patients* ***(B)****, and pretreated patients* ***(C)*** *according to poor adherence* (triangles) and good adherence (squares) to therapy. Diagonal dashed line represents maximal virologic response (y = log (40)-x).

Discussion

In this study the twenty-four weeks results of treatment of HIV-1 infected children with highly active antiretroviral therapy (HAART) consisting of indinavir, zidovudine and lamivudine are reported. A good clinical response was seen in all children. No serious invasive or opportunistic infections occurred since the initiation of triple therapy. Impaired growth was seen in 81% of the children at baseline and responded to therapy in 76% of the children with impaired growth at baseline. As growth seems to be one of the most sensitive indicators of disease progression in children and weight gain may be another indicator of antiretroviral therapeutic efficacy, growth is an important parameter. (27-30)Our results show that in spite of the specific problems encountered in treatment of HIV-1 infected children, i.e. possible differences in pharmacokinetics and pharmacodynamics between adults and children, the relative immaturity of the immune system in young children, and specific pediatric issues concerning therapy compliance, HAART can be used as successfully in children as in adults. Since we observed substantial interindividual differences in the pharmacokinetics of indinavir in children (manuscript in preparation), we propose to measure indinavir pharmacokinetics (and possibly also pharmacokinetics of other protease inhibitors) to determine the individual dosage necessary for an area under the curve which is comparable with adult values. Combination therapy reduced the plasma HIV-1 RNA load to less than 500 copies/ml in

70% of the children and to less than 40 copies/ml in 48% of the children within six months of therapy. Viral load reductions were observed to less than 500 copies/ml in 95% of the patients at month six and to less than 40 copies/ml in 68% of the children at month six after exclusion of patients with documented poor compliance. These results are comparable to results obtained in adults. Gulick et al. reported a decline of the viral load to less than 500 copies/ml in 90% of the patients at week 24 with the same HAART regimen. (1) Our results are particularly reassuring considering the fact that no preselection of patients was made on the basis of expected problems with compliance. The virologic response rate in this study is better than the one obtained by a combination of zidovudine and lamivudine in children, which resulted in a viral load less than 500 copies/ml in only 46% of the patients after six months. (2) Indinavir alone gave less pronounced reductions in viral load than indinavir in combination with zidovudine and lamivudine, with a maximum decrease of 1.35 $\log_{10}$ after 24 weeks with a dosage of 1500 mg/m^2/day. These reductions could not be sustained, since after 8 weeks of indinavir monotherapy viral load increased to approximately 0.5 $\log_{10}$ copies/ml below baseline. (14) Data from other studies with HAART consisting of a combination of a protease inhibitor and two reverse transcriptase inhibitors in children are limited. With median declines of the viral load varying from 0.6 $\log_{10}$ to 2.3 $\log_{10}$ after 24 weeks of therapy none of these studies describes a decline of the viral load comparable to the median decline of this study (2.8 $\log_{10}$). (4, 16-20)

Besides the marked reductions of plasma RNA load, a significant increase of CD4 cell count was seen with a median relative increase from 79% of the lower limit of the reference value to 106% after 6 months of therapy. Plasma HIV-1 RNA and CD4 cell count are both surrogate markers, but recent studies in children showed that plasma HIV-1 RNA level and CD4 lymphocyte counts are independent predictors of clinical course. As in adults a linear, age-independent relationship exists

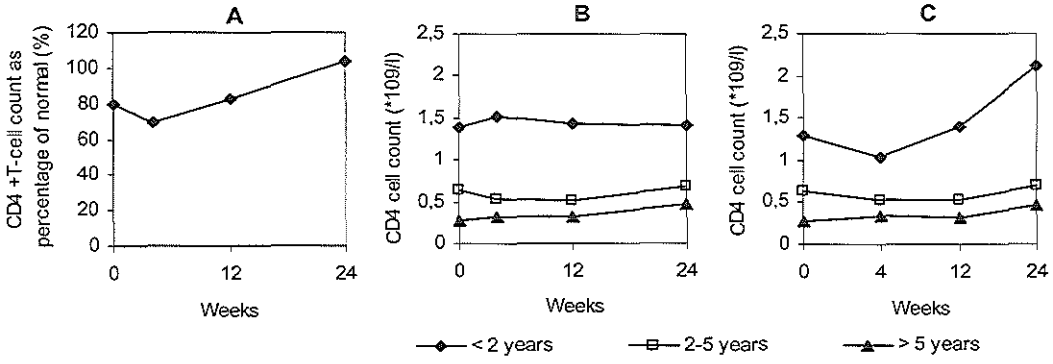

Figure 3: A, *Median relative CD4+ T-cell counts of all patients in relation to the lower limit of the age-specific reference value.* ***B,*** *Median absolute CD4+T-cell counts of all patients. Patients are divided into 3 groups in accordance with age-related differences in reference values.* ***C,*** *Median absolute CD4+ T-cell counts of 19 patients who completed therapy without documented poor adherence. Patients are divided into 3 groups in accordance with age-related differences in reference values.*

between $\log_{10}$ plasma RNA and relative risk of disease progression, which strongly supports therapeutic efforts to achieve plasma virus levels as low as possible. (6)

The combination of indinavir, zidovudine and lamivudine was generally well tolerated. In 59 % of the patients no adverse events were seen. Adverse events were mild and mainly of gastro-intestinal origin. Hematuria that resolved under adequate hydration and a short discontinuation of the indinavir was seen in only three patients. In contrast to adults where in 12% of the patients clinical nephrolithiasis was reported (1), no nephrolithiasis was seen.

In children special attention is needed to achieve good compliance in the ingestion of antiretroviral drugs. Even under study conditions, eight of twenty-seven patients (29%) had problems with the strict regimen, which resulted in less reduction in viral load. In order to facilitate compliance, a pediatric formulation of indinavir is urgently needed.

As low plasma concentrations of indinavir are a major and independent risk factor for virologic treatment failure (31), calibration of the pharmacokinetics of indinavir (data not shown) to adequate adult values makes this study unique and may be partial responsible for the good results. Although our results have not been obtained by a controlled trial, comparison with previous studies with indinavir monotherapy and therapy with two reverse transcriptase inhibitors makes it highly unlikely that these therapies would have resulted in comparable results.

Taken together, our data show that combination therapy consisting of a protease inhibitor and two reverse transcriptase inhibitors is as efficacious and generally well tolerated in children as in adults.

Dutch study group for children with HIV-1 infections

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Two year treatment results using protease-inhibitor containing antiretroviral therapy in Dutch HIV-1 infected children

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Chapter 10

Abstract

Clinical, virological and immunological responses to treatment with either indinavir or nelfinavir and zidovudine and lamivudine were determined in 32 HIV-1 infected children who participated for at least 96 weeks in an prospective, open, uncontrolled multicenter trial. The pharmacokinetics of indinavir or nelfinavir were determined and showed large interindividual differences. After 96 weeks of therapy, 69% and 50% of the patients had an HIV-1 RNA load below the detection limit of 500 and 40 copies/ml respectively. Virological failure was associated with poor compliance and younger age (independent of baseline viral load and pretreatment). Relative CD4 cell counts in relation to the median of the age-specific reference value increased significantly from a median value of 44% at baseline to 94% after 96 weeks. In a high percentage of the children clinical, virological and immunological response rates to combination therapy were optimal during the initial two years of therapy.

Introduction

Combination therapy consisting of one protease-inhibitor and two nucleoside-analogues has yielded impressive long-term clinical, virological and immunological improvements in HIV-1 infected adults. (1-6) However, in most pediatric studies virological response rates to HAART are inferior to those in adults. (7-15) Since virological suppression is associated with long-term success of HAART, this may have major clinical implications for the future health of these children. (16-18)

The institution of optimal treatment strategies in HIV-1 infected children poses a enormous challenge. First, large interindividual differences of pharmacokinetics of antiretroviral drugs, especially of protease inhibitors, complicate an optimal dosing of antiretroviral drugs in children. Secondly, it is difficult to maintain adherence to combination therapy during the years following the initiation of HAART. Problems such as the intake of evening medication during sleeping time or afternoon medication during schoolhours, unwillingness of young children and adolescents to take medication and poor palatability and side-effects of medication have to be dealt with.
To date, there are no published data on the long-term follow-up (96 weeks) of HIV-1 infected children, who are treated with HAART.

We here report the 96 weeks results of a prospective, open, uncontrolled, multicenter study on the clinical, immunological and virological response to therapy with either indinavir, zidovudine, and lamivudine or nelfinavir, zidovudine and lamivudine in 32 children with HIV-1 infection.

Methods

PI naive HIV-1 infected children with a viral load above 5000 copies/ml and/or a CD4 cell count below their age-specific reference value were included.

The study protocol was approved by the medical ethical committees of all participating centers. Written informed consent was obtained from parents or legal guardians. Patients' medical histories, physical examination and laboratory analyses were analyzed twice before the initiation of the medication and after 1, 2, 4, 8, 12, 24, 36, 48, 60, 72, 84 and 96 weeks. (3) Lymphocyte subsets were analyzed with the FACSCount System (Becton Dickinson Immunocytometry Systems,

San Jose, CA, USA).(25) Plasma HIV-1 RNA quantitation was analyzed by an *in vitro* (-PCR) test (most samples: Amplicor HIV-1 monitor test version 1.5 Roche Diagnostic Systems and a few samples: NASBA assay, nuclisensHIV-1 RNA; Organon Teknika Boxtel, The Netherlands) with a lower limit of detection of 500 and 400 copies/ml. Plasma samples with an HIV-1 RNA <500 copies/ml were subsequently tested with an ultrasensitive Amplicor HIV-1 monitor test with a detection limit of 40 copies/ml.(26)

Medication and pharmacokinetics

Medication was prescribed in the following doses: indinavir 400 mg/m^2 q8h or nelfinavir 30 mg/kg q8h, zidovudine 120 mg/m^2 q8h, lamivudine 4 mg/kg q12h. When patients were too young to swallow capsules, indinavir capsules were opened by the caregivers and were dissolved in five to ten milliliters of water. The importance of food restrictions (administration of indinavir one hour before or two hours after meals and administration with a meal) was repeatedly emphasized. A day to day medication scheme including the times with food restrictions was given.

At week 4, patients were admitted to determine the steady state pharmacokinetics of indinavir or nelfinavir. When a dosage adjustment of indinavir or nelfinavir was necessary to normalize the area under the curve-concentration (AUC) curve to adult values (indinavir: 20 mg/L*hr, range 10-30 mg/L*hr, nelfinavir: 13-20 mg/L*hr), this procedure was repeated.

Compliance

Drug- compliance was assessed by interviews of the parents, by measurement of indinavir or nelfinavir plasma levels and by medication diaries. The coaching of parents and children was intensified, when problems with compliance were encountered. When the interviews of the children or their parents, the medication diaries and/or plasma levels of indinavir or nelfinavir showed that serious (i.e. more than one time) problems with adherence to the medication scheme existed, these children were considered to be poorly compliant. Patients with good compliance were defined as those in whom according to the interviews, the medication diaries and the plasma levels of indinavir or nelfinavir no problems (or only problems at one visit) were found.

Adverse Events

Adverse events were defined as any grade clinical sign or symptom, or meaningful laboratory-test abnormality, excluding disorders associated with HIV-1 infection. All adverse events were rated according to severity and relation to study drug.

Statistical Analyses

Children who started with therapy at least 96 weeks before the time that analysis was performed were included. In the analysis of the median change from baseline, viral loads of patients below the detection limit were considered to be 40 copies/ml. At each time point (week) we calculated the percentage of patients from which the values were below the detection limit (500 or 40 copies/ml). For occasional missing data in the intention to treat analysis, we considered the response value as greater than the detection limit concerned. Relative CD4 cell counts in relation to the median of the age-specific reference value (27) were calculated by dividing the individual value at the different time points by the median of the reference value. The calculation of CD4+ T-cell counts in relation to

the median of the age-specific reference value results in an independent parameter for the degree of CD4+ T-cell restoration. Because absolute CD4+ T-cell counts are highly dependent on the age of the patients, the magnitude of these changes depends on the age distribution of the cohort. In order to solve this problem, the number of CD4+ T-cells is commonly presented as a percentage of the total T-cell count. However, this parameter is influenced by the major changes in the number of CD8+ T-cells which are observed in HIV-1 infected patients.

Changes of CD4 cell counts were evaluated using the Wilcoxon signed rank test. The relation between the plasma HIV-1 RNA levels at week 96 and various characteristics (baseline viral load, prior treatment, compliance and age) was investigated using multiple linear regression analysis for normally distributed data. In this analysis both viral load results were logarithmically transformed, and allowed for the fact that various children at week 96 had an HIV-1 RNA load which was actually lower than the detection limit (i.e. left-censored data). (28) All p-values are two-tailed.

Z-scores to evaluate growth were used to express the deviation (in standard deviation (SD) units) from the Dutch reference curves for age and gender. (7, 8)

Results

Thirty-two HIV-1 infected children were enrolled at 5 centers. Baseline characteristics of the thirty-two children are depicted in Table 1. The median age of the children was 5.4 years (range: 3 months to 16.4 years), seventeen patients were not previously treated and fifteen had received prior treatment with nucleoside analogues (mostly zidovudine monotherapy). The median HIV-1 RNA level was 136,385 copies/ml (range: 2,680-761,500). In table 2 the number of patients using either indinavir or nelfinavir at each time point are presented and if applicable the reason of switch or stop is mentioned. Twenty-seven patients started with an indinavir containing regimen and five children started with a nelfinavir containing regimen. Eight children were too young to swallow capsules and received indinavir dissolved in water.

In eight children, indinavir was switched to nelfinavir after a median period of 48 weeks (range 2-72). In six children the medication was changed because of virological failure, in two other children because of toxicity (interstitial nephritis) and because of reluctance to swallow indinavir dissolved in water. In one child indinavir was switched to indinavir/ritonavir (500/100 mg/m2 q8h) after 60 weeks, because of virological failure. At week 72 indinavir/ritonavir was changed into nevirapine because of an interstitial nephritis. Five children were lost to follow-up (after 24, 24, 60, 60 and 60 weeks).

Twenty children (62%) were defined as children with a good compliance and twelve children met the criteria for poor compliance (38%).

Pharmacokinetic results

In eight patients a dose of 400 mg/m^2 q8h of indinavir was adequate. In one patient indinavir was discontinued before pharmacokinetics were performed. In 18 of 26 patients (69%) the dose of the indinavir was increased in order to achieve AUC-values between 10 and 30 mg/L*hr. In eight patients the dose had to be increased to 500 mg/m^2 q8h, in one patient to 550 mg/m^2 q8h, in six patients to 600 mg/m^2 q8h, in one patient to 660 mg/m^2 q8h and in two patients to 800 mg/m^2 q8h. Large interindividual differences in the pharmacokinetics of indinavir have been observed and have been presented in more detail. (9)

Five children started with nelfinavir. In three children adequate AUC values of nelfinavir were achieved with doses of 17, 24 and 27 mg/kg q8h. In two children the dosage was increased from 30 mg/kg q8h to 40 and 45 mg/kg q8h.

Table 1: Baseline characteristics of the study patients

Median age in years (range)					5.4 (0.4-16.4)			
Sex (male/female)					18/14			
Race/ethnicity								
White					3			
Non-white					29			
Route of transmission								
Vertical					23			
Blood products					4			
Unknown					5			
Clinical stage (CDC classification)*								
N1	2	A1	3		B1	1		
N2	3	A2	5		B2	5	C2	2
N3	1	A3	4		B3	1	C3	1
No prior treatment					17			
Prior treatment with								
Zidovudine					11			
Zidovudine/zalcitabine					1			
Zidovudine/didanosine					1			
Zidovudine/lamivudine					1			
Zidovudine/lamivudine/didanosine					1			
HAART								
IDV/ZVD/3TC					27			
NFV/ZDV/3TC					3			
NFV/d4T/ddI					2			
Median copies of HIV-1 RNA/ml plasma (range)					136,385	(2,680-761,500)		
Naive to antiretroviral therapy					250,000	(23,900-761,500)		
Experience with antiretroviral therapy					29,300	(2,680-718,000)		

*Clinical and Immunologic categories as defined by the US Centers for Disease Control and prevention (CDC). (37)

Clinical results

Serious invasive or opportunistic infections were only seen in one patient with poor compliance. The BMI Z score increased significantly (p=0.05) during the follow up period from a median (interquartile range (IQR)) of –0.5 (-1.4 to 0.6) at baseline to 0.2 (-0.7 to 0.6) after 96 weeks of HAART. The median of height Z scores did not increase significantly, but height Z scores of fifteen (47%) individual children did increase during 96 weeks of HAART (data not shown). In the other children Z scores decreased.

Table 2: the distribution of the numbers of patients among the protease inhibitors at each time point.

Week	IDV N =	NFV n =		IDV/ RTV n =		NVP n =		NFV/ RTV n =		Stop/lost to follow up n =		Reason switch
0	27	5	A15, A16, A20, B04, B05									
1	27	5										
2	26	6	+A24									Taste IDV
4	26	6										
8	26	6										
12	26	6										
24	21	8	+A19, +B03	1	+E02					2	+A15 -E01	A19: virological failure, B03: wish of the patient, E02: virological failure, A15: moved to other country, E01: lost to follow up
36	20	10	+A17, +E02	0	-E02					2		A17: virological failure, E02: possible toxicity
48	19	11	+A04							2		A04: virological failure
60	15	11		1	+A11					5	+A01, +D01 - C01	A11: virological failure, A01: death, D01: severe psychological problems, C01: lost to follow up
72	13	12	+A07, +A22, -A19	0	-A11	1	+A11	1	+A19	5		A07: toxicity, A22: virological failure, A11: toxicity, A19: virological failure
84	13	12		0		1		1		5		
96	13	12		0		1		1		5		

HIV-1 virologic responses

The levels of the viral load after start of HAART in children with good compliance and children with poor compliance are demonstrated in Figure 1A and 1B. The median (IQR) changes from baseline of $\log_{10}$ HIV-1 RNA in all patients were -2.0 (-2.3 to -1.8) at week 4, -2.7 (-3.0 to -1.8) at week 12, -2.7 (-3.1 to -1.8) at week 24, -2.7 (-3.2 to -2.0) at week 48 and -2.6 (-3.6 to -1.8) at week 96. Figure 2 shows the proportion of patients whose viral load levels decreased to less than 500 and less than 40 copies/ml in an as treated and an intention to treat (missing value equals failure) analysis. In children with a good compliance the percentages of children with an HIV-1 RNA below the detection limit of 500 and 40 copies/ml were higher compared to the children with a poor compliance: at week 48 90% and 56% versus 42% and 1% respectively and at week 96 85% and 65% versus 1% and 0% respectively.

Multiple regression analysis of the parameters compliance, pretreatment, baseline viral load and age showed that compliance and age were significantly related with viral load. In patients with a poor compliance the $\log_{10}$ HIV-1 RNA was 2.5 ($p<0.001$) higher than in patients with an adequate

compliance. For age the $\log_{10}$ HIV-1 RNA at week 96 decreased 0.12 (p=0.02) for each year. Baseline viral load and prior treatment did not significantly correlate with the plasma HIV-1 RNA at week 96.

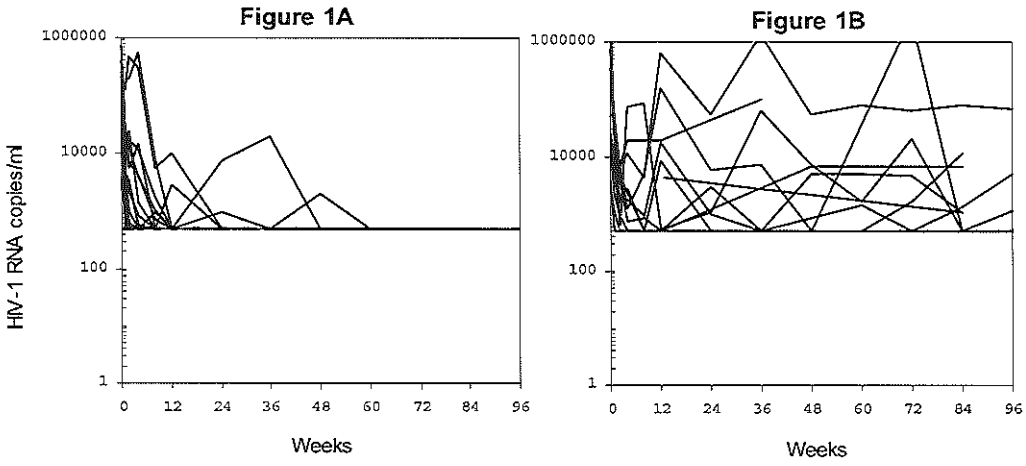


Figure 1A: *Levels of HIV-1 RNA in 20 patients who completed therapy without documented poor compliance.*
Figure 1B: *Levels of HIV-1 RNA of the 12 patients with documented poor compliance.*

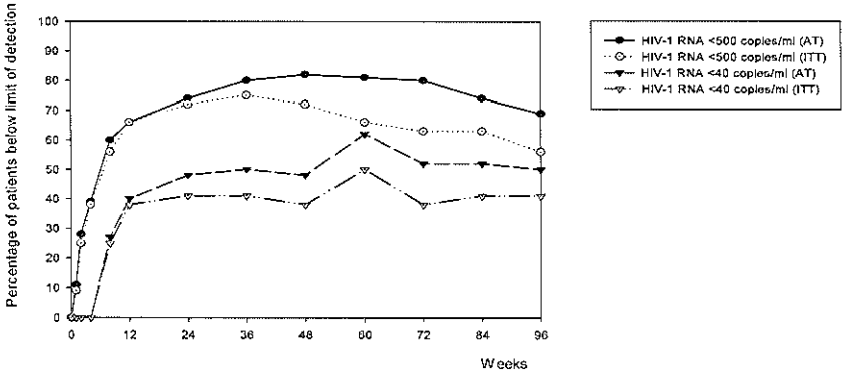


Figure 2: *The proportion of patients whose viral load levels decreased to less than 500 and less than 40 copies/ml in an as treated and an intention to treat analysis.*
AT: As Treated analysis in which only children who were receiving treatment were included.
ITT : Intention To Treat (Missing value = Failure) analysis in which HIV-1 RNA levels were assigned a value of more than 500 or 40 copies/ml for all missing values, including visits after discontinuation of treatment.

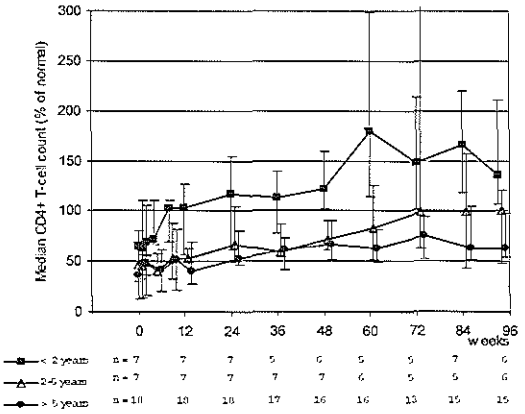
CD4 cell counts

Figure 3A shows relative CD4+ T-cell counts of patients in three different age groups (<2 years, 2-5 years and > 5 years). In the group of thirty-two patients the median relative CD4 cell count (IQR) was 44% (29-68) at the start of the therapy and 72% (54-109) after 48 weeks. After 96 weeks a significant ($p< 0.001$) increase to a median of 94% (54-116) was observed. Figure 3B shows the absolute CD4 cell counts in three different age groups (<2 years, 2-5 years and > 5 years). The median absolute CD4+ T-cell count (IQR) of all patients was 545 x 10^6 cells/L (285-903) at baseline, 895 x 10^6 cells/L (493-1505) after 48 weeks and 980 x 10^6 cells/L (493-1402) after 96 weeks of treatment ($p= 0.002$).

After 72 weeks CD4+ T-cell counts reach a plateau, which is equivalent to the CD4+ T-cell counts in healthy children. In children younger than 2 years absolute CD4+ T-cell counts even decreased after 72 weeks, reflecting the normal age-related strong decline of CD4+ T-cell counts in children at this age.

The change of the CD4+ T-cell count as percentage of normal from baseline was not significantly different between the age groups at any time-point.

The immunologic reconstitution (absolute CD4+ T-cell counts, CD4+ T-cell counts as percentage of normal, CD4+ T-cell counts as percentage of total T-cell count) was not different at any time-point in virologic responders (HIV-1 RNA at week 48 < 500 copies/ml) and virologic non-responders.



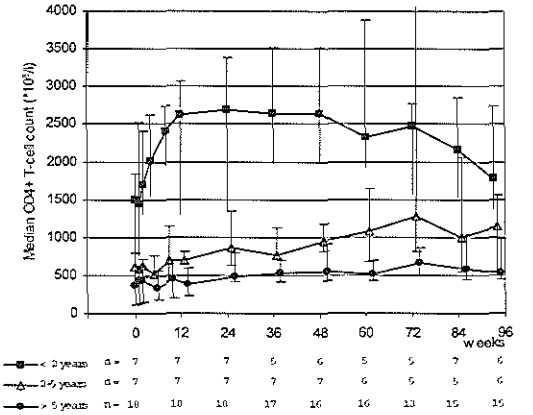


Figure 3A: *Median relative (% of normal) CD4 cell counts of all patients. Patients are divided into three groups according to age related differences in reference values*

Figure 3B: *Median absolute CD4 cell counts of all patients. Patients are divided into three groups according to age related differences in reference values*

Adverse Events

Although seventeen (53%) out of the thirty-two patients had adverse events, combination therapy was well tolerated by almost all children. The majority of the adverse events were mild and occurred during the initial phase of the treatment. The most common indinavir related side-effects were: diarrea (n= 6), vomiting (n=6), loss of appetite (n=5), headache (n=3), abdominal pain (n=4) and

hematuria (n=5), which resolved after discontinuing medication for two days and re-emphasizing the importance of adequate fluid intake. In two children, indinavir was switched to another antiretroviral agent (one to nelfinavir and one to nevirapine) because of an interstitial nephritis after 72 weeks. The renal function and renal ultrasounds normalized after the switch. Two children had nelfinavir related side-effects: diarrea (n=1) and loss of appetite (n=1).
Clinically overt lipodystrophy was not observed.

To compare the frequency of indinavir and nelfinavir related side effects, the number of episodes with one or more adverse events and the total number of weeks that patients used indinavir or nelfinavir were calculated. 0.4 indinavir related episodes with side effects per patientyear were observed versus 0.16 nelfinavir related episodes with side effects per patientyear.

Discussion

In this study the long-term clinical, virological and immunological response to HAART, consisting of one protease inhibitor (mostly indinavir) and two nucleoside analogues, was evaluated in thirty-two HIV-1-infected children. Although twelve of thirty-two patients (38%) had problems with adherence to the regimen, a good clinical response was seen in all children. New serious invasive or opportunistic infections only occurred in one patient with poor compliance. The BMI Z score increased significantly during the follow up period. The median of height Z scores did not increase significantly, but height Z scores of fifteen (47%) individual children did increase during 96 weeks of HAART. Since growth seems to be one of the most sensitive indicators of disease progression in HIV-1 infected children, growth is an important parameter. (10-13)

Analyses of the virological response data showed that these are consistent with the previously observed finding that a similar rate of suppression of HIV replication can be obtained in children during the first year of treatment (1, 2, 6, 14) as compared to adults (15, 16). In addition, we demonstrated that virological suppression can be retained by the majority of the children during 96 weeks follow-up. In 69% of the children HIV-1 RNA was below the detection limit of 500 copies/ml and in 50% of the children below the detection limit of 40 copies/ml after 96 weeks. Even in the conservative intention-to-treat analysis in which missing values were assigned a value of more than 500 or 40 copies/ml, 56% and 41% of the children had a viral load below the detection limit of 500 and 40 copies/ml respectively. In contrast to the percentage of patients with a viral load <40 copies/ml, the percentage of patients with a viral load <500 copies/ml decreased during the second year of follow up. This indicates that in children with a viral load between 500 and 40 copies/ml long-term virological outcome is worse compared to children with a viral load <40 copies/ml. Raboud et al have reported similar observations in adults. (17, 18) It remains to be studied, whether a suboptimal virological response rate two years after initiation of HAART is associated with a decreased long-term clinical and immunological response rate.

The virological results in our study are less good than those obtained in adults. Gulick et al. reported a decline of the viral load to less than 500 copies/ml in 78% of the patients and to less than 50 copies/ml in 66% of the patients at week 96 with indinavir, zidovudine and lamivudine. (16) However, it is difficult to compare data from a their study-population with data from this cohort in which no selection on the basis of expected compliance was made. The virological response rate in our study (69% <500 copies/ml after 96 weeks) is high as compared to those in most pediatric

studies. (19-27) All four studies which optimized the dosage of PI's on the basis of pharmacokinetic analyses showed superior virological response rates compared to those in which standard dosages were used. (1-3, 5) Interindividual pharmacokinetic differences result in inadequate plasma PI levels in a part of the children treated with a fixed dose. (3, 9, 28-31) Since virological response is associated with plasma protease inhibitor levels, inadequate plasma PI levels may be partly responsible for the differences in virological response rates. (9, 28, 29, 32, 33) We therefore advise to measure PI levels in all children treated with a PI to reach optimal AUC levels as have been determined in adults.

The inferior virological results which have been reported in HIV-1 infected children treated with HAART (19-27) may be a reflection of the challenges which are encountered in the treatment of children with HIV-1/AIDS. Difficulties with adherence and with pharmacokinetics of protease inhibitors in children demand an intensive, child adjusted approach. In addition, inferior virological results in children have also been attributed to high initial viral loads in children. Children tend to have higher baseline viral loads than adults (34), which have been associated with a smaller reduction in viral load (2, 14, 25). Interestingly, we found no correlation between baseline HIV-1 RNA and virological response. However for age and virological response there was a significant correlation in a multivariate analysis. Younger children have significantly less viral load reduction regardless of compliance, pretreatment and baseline viral load. Due to the small sample size interpretation of this finding is difficult.

CD4+ T-cell counts increased significantly after the initiation of the therapy from 545 x 10^6 cells/L (285-903) at baseline to 980 x 10^6 cells/L (493-1402) after 96 weeks of treatment ($p= 0.002$). The median CD4 cell count as percentage of normal (IQR) was 44% (29-68) at the start of the therapy and after 96 weeks a significant ($p< 0.001$) increase to a median of 94% (54-116) was seen. After 72 weeks of therapy CD4+ T-cell counts reached a plateau, which is equivalent to the CD4+ T-cell counts in healthy children.

Immunologic reconstitution was observed to same degree in virologic responders (HIV-1 RNA at week 48 < 500 copies/ml) and virologic non-responders.

The HAART regimens were generally well tolerated. In 47 % of the patients no adverse events were seen. The majority of the adverse events occurred during the initial phase of the treatment. They were mild and mainly of gastro-intestinal origin. Hematuria which resolved under adequate hydration and a short discontinuation of the indinavir was seen in five patients. In two patients an interstitial nephritis occurred after 72 weeks of therapy. Nephrolithiasis was not observed in contrast to the situation in adults where clinical nephrolithiasis was reported in 12% of the patients. (35) Since elevated plasma concentrations of indinavir have been associated with urological complications (36), calibration of the pharmacokinetics of indinavir (data not shown) to adequate adult values may have contributed to the low incidence of urological symptoms.

Indinavir related side-effects seem to occur more frequently than nelfinavir related side-effects (0.4 versus 1.6 episodes per patientyear) However, this study was not designed to compare nelfinavir and indinavir containing regimens.

This study emphasis the need for special attention in children to achieve good compliance in the ingestion of antiretroviral drugs. Even under strict study conditions, twelve of thirty-two patients (38%) had problems with the regimen, which resulted in a decreased reduction of the viral load. A simplified dosing regimen may contribute to achieve an improved compliance. In conclusion,

our data show that in a high percentage of the children clinical, virological and immunological response is optimal after two years of therapy.

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Immune reconstitution in HIV-1 infected children treated with HAART is independent of their age and pretreatment immunestatus

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Chapter 11

Abstract

Objective: To evaluate long-term immune reconstitution of children treated with HAART. *Design:* Two prospective, open, uncontrolled national multicentre studies. *Methods:* We studied the long-term immunological response to HAART in 71 HIV-1-infected children (age: 1 month to 18 years). Blood samples were taken before and after HAART was initiated with a follow-up of 96 weeks. Lymphocyte immunophenotyping for peripheral CD4+ T-cells, CD8+ T-cells, naive and memory subsets was performed on whole blood samples. Relative CD4+ - and CD8+ T-cell counts were calculated in relation to the median of the age-specific reference. *Results:* The absolute CD4+ T-cell count, CD4+ T-cell percentage and CD4+ T-cell count as percentage of normal increased significantly ($p<0.001$) to a median of 939 x 10^6 cells/L (range 10-3520), 32% (range 1-50%) and 84% (range 1-161%) respectively after 48 weeks. This increase was predominantly caused by naive CD4+ T-cells. We found a correlation between the increase of absolute naive CD4+ T-cell counts and age. However, when CD4+ T-cell restoration was studied as percentage of normal values, the inverse correlation between the increase of naive CD4+ T-cell count and age was not observed. In addition, no difference in immunologic reconstitution was observed at any time-point between virologic responders and non-responders. *Conclusions:* Normalisation of the CD4+ T-cell counts in children treated with HAART is age-independent, indicating that children of all age-groups can meet their CD4+ T cell production demands. In general, it appears that children restore their CD4+ T-cell counts better and more rapidly than adults, even in a late stage of HIV-1 infection.

Introduction

Highly active antiretroviral therapy (HAART) has only recently been applied in children infected with HIV-1. The use of HAART leads to a reduction in plasma HIV-1 RNA loads to undetectable levels in a high percentage of these children and results in a significant recovery of CD4+ lymphocyte counts. (1-6)

In adults immune reconstitution following HAART shows a biphasic pattern consisting of an initial rapid redistribution of memory cells and a gradual slow increase in naive T-cells. (7, 8) Children with HIV-1 infection have a greater capacity to reconstitute their naive CD4+ T-cells when compared to HIV-1-infected adults treated with similar antiretroviral therapy. (6) This is not an unexpected finding, since naive T-cell recovery is believed to be thymus-dependent and thymic function diminishes with age. (9, 10) Increased production of naive cells is associated with thymic enlargement in children treated with anti-cancer chemotherapy as well as in children treated with HAART as shown on radiographs or by magnetic resonance imaging. (9, 11, 12)

CD4+ T-cell numbers in HIV-1-infected children on HAART recover more rapidly than CD4+ T-cells in HIV-1-infected adults. (6, 11, 13-16) However, it is still unclear to what extent the number of CD4+ T-cells of HIV-1-infected children is capable to normalise, since data on long-term T-cell dynamics in HIV-1-infected children on HAART are not available. In a considerable number of HIV-1-infected adults treated with HAART, CD4+ T-cell numbers stabilised or even slightly decreased after 1½ years of therapy, sometimes without having reached normal levels. (17) We evaluated the long-term immune reconstitution in HIV-1-infected children who were treated with HAART, consisting of one protease inhibitor and two nucleoside reverse transcriptase inhibitors during a period of 96 weeks. We analysed changes in the number of CD4+ T-cells, CD4+ T-cell naive and memory subsets

and CD8+ T-cell counts and compared those to the normal values that were previously reported for the different age groups. (18, 19)

In addition to the quantitative analyses, T-cell function after stimulation with CD3 mAb plus CD28 mAb was analysed.

Methods

Patients

Seventy-one HIV-1-infected children were enrolled in two prospective, open, uncontrolled, studies to evaluate the clinical, immunological and virological response to combination therapy with either indinavir, zidovudine and lamivudine or nelfinavir, stavudine and lamivudine. Inclusion and exclusion criteria for these studies were equal and have previously been described in detail. (3) Children 1 month to 18 years of age, with or without prior treatment with reverse transcriptase inhibitors, and a viral load of more than 5000 copies/ml or a CD4+ T-cell count less than the lower limit of the age-specific reference value were eligible for enrolment. Study protocols were approved by the medical ethical committee of all participating institutions. Written informed consent was obtained from the parents or the legal guardian. Blood samples were taken before HAART was started (2-0 weeks prior to initiation of the study) and 1, 2, 4, 8, 12, 24, 36, 48 and 96 weeks after the initiation of combination therapy. Medication was given in the following doses: indinavir 400 mg/m^2 q8h, nelfinavir 30 mg/kg q8h, zidovudine 120 mg/m^2 q8h, lamivudine 4 mg/kg q12h and stavudine 1 mg/kg q12h. Multiple dose pharmacokinetics of either indinavir of nelfinavir were determined four weeks after HAART was initiated. A dosage adjustment of indinavir or nelfinavir was done when necessary to adjust the area under the time concentration (AUC) curve to adult values.

Immunophenotyping and T-cell function in vitro

Lymphocyte immunophenotyping of peripheral CD4+ and CD8+ T cells was performed on lysed whole blood samples by flow cytometry using triple staining. Lymphocytes were phenotyped as naive and memory CD4+ and CD8+ T-cells by three-color immunofluorescence flow cytometry using combined staining with CD45RA and CD 62L (L-selectin) or CD27 monoclonal antibodies (mAb). T cells expressing both CD45RA and CD62L or CD27 were considered truly naive cells, whereas cells, which lacked either CD45RA and CD62L or CD27 were regarded as memory cells. (20, 21) Previously, it has been demonstrated that naive and memory subset distribution as measured with either CD45RA and CD62L mAb or CD45RA and CD27 mAb yielded identical results. (22)

T-cell function was determined in whole-blood lymphocyte culture (23). Proliferative responses to CD3 mAb plus CD28 mAb were measured after 4 days of culture by means of the incorporation of ^{3}H-thymidine added 24 hours before harvest. (24) Proliferative capacity was calculated as counts per minute (CPM) per 10^3 CD3+ T-cells and results are expressed as percentage of the median of 3450 healthy adult donors (24).

Plama HIV-1 RNA determination

Plasma HIV-1 RNA levels were measured by an in vitro nucleic acid amplification test (AMPLICOR HIV-1 MONITOR Test (Roche Diagnostic Systems) with a lower limit of quantification of 500 copies/ml, by the NASBA assay (Nuclisens HIV-1 RNA; Organon Teknika, Boxtel, The Netherlands)

with a lower limit of quantification of 400 copies/ml or by the Quantiplex b DNA test (Bayer, The Netherlands) with a lower limit of quantification of 50 copies/ml. The test used at baseline was also used at every follow-up visit. The on-treatment-analysis method was used to calculate percentages of patients with an HIV-1 RNA below the lower limit of quantification (LLQ) of 500 copies/ml.

Statistical analysis

All patients with analyses made at baseline (in ten patients in which baseline values were missing, analyses at week 1 were considered as baseline) and at least 12 weeks of follow-up were included. SPSS 9.0 (SPSS, Chicago) was used for statistical analysis. Because absolute CD4+ T-cell counts are highly dependent on the age of the patients and on the stage of disease, relative CD4+ T-cell counts (total CD4+ T-cells and naive CD4+ T-cell counts) in relation to the median of the age-specific reference values (18, 19) were calculated by dividing the individual value at the different time-points by the median of the reference value at the different time-points. Results are expressed as percentage of normal. Since CD4+ T-cell percentage of total T-cells is influenced by changes in the number of CD8+ T-cells, calculation of CD4+ T-cell counts as percentage of normal, results in a parameter that is independent of CD8+ T-cell count. Correlation coefficients were obtained by Spearman rank correlation. Differences between paired variables were analysed with the Wilcoxon signed ranks test and between groups with the Mann-Whitney *U* test. All p-values are two-tailed.

Results

Baseline characteristics of the seventy-one patients are presented in Table 1. Forty-two of 71 (59%) patients were not pre-treated and 29 of 71 (41%) patients had received prior treatment with nucleoside reverse transcriptase inhibitors (mostly with zidovudine monotherapy for an average of 32 months). Thirteen children with prior zidovudine therapy were placed on the stavudine arm, whereas the other sixteen children with prior zidovudine therapy continued zidovudine. Baseline viral load and prior treatment did not significantly correlate with the plasma HIV-1 RNA at week 96. The change from baseline in absolute CD4+ T-cell count, CD4+ T-cell count as percentage of total T-cells and CD4+ T-cell count as percentage of normal was not significantly different between children with or without prior treatment at all time points. Thirty-seven children completed 96 weeks of follow-up. Four children were lost to follow-up, one child died and the other twenty-nine children who did not complete 96 weeks of follow-up entered the study-cohort shorter than 96 weeks before the time of analysis.

Plasma HIV-1 RNA

The medians and interquartile ranges (IQR) of the viral load after start of HAART and the percentages of patients with a viral load below the LLQ in responders and non-responders are depicted in Figure 1. Virologic responders were defined as those who either reached an undetectable viral load (<500 or <400 copies/ml) or had a > 1.5 log reduction in viral load compared to baseline at week 12 after the initiation of HAART which was maintained during the follow-up period. Fifty-six patients were qualified as responders and fifteen as non-responders. In the virological responders, HIV-1 RNA was below the LLQ in 79% of the children after 12 weeks and in 91% after 48 weeks.

Thirty-seven children completed 96 weeks of follow-up, thirty-one of these children were classified as responder and six as non-responder.

Table 1: Baseline characteristics of the study patients

Characteristic				
Number of patients	71			
Age in years*	5.1 (0.1-17.5)			
Route of acquisition:				
Vertical	58			
Blood products	6			
Heterosexual	3			
Unknown	4			
Clinical stage (CDC-classification)†:	A1 6	B1 2	C1 3	
N1 1	A2 13	B2 6	C2 12	
N2 5	A3 3	B3 5	C3 14	
N3 1				
HAART				
IDV/ZVD/3TC	32			
NFV/d4T/3TC	26			
NFV/ZDV/3TC	11			
NFV/d4T/ddI	2			
Median HIV RNA (copies/ml) *	82,000 (620-27,000,00)			
T -cells absolute (10^6 cells/L) *	All age groups	< 2 years	2-5 years	≥ 5 years
Number of patients	n = 71	n = 18	n = 17	n = 36
CD4+ T-cells	471 (0-3580)	767 (81-3580)	561 (2-1490)	370 (0-1140)
Naive‡ CD4+ T-cells	211 (0-2880)	967 (47-2880)	226 (0-1237)	167 (0-616)
Memory§ CD4+ T-cells	205 (1-2613)	570 (5-2613)	229 (2-426)	193 (1-646)
% of total T cells	17 (0-60)	22 (7-60)	17 (0-51)	17 (0-51)
% of normal	41 (0-143)	31 (3-143)	43 (0-115)	41 (0-114)
CD8+ T-cells	1147 (60-7369)	1680 (640-5436)	1070 (60-7360)	1030 (150-4860)
Naive CD8+ T-cells	257 (17-1312)	753 (363-1312)	246 (17-883)	188 (26-773)
Memory CD8+ T-cells	800 (24-6477)	734 (166-4131)	744 (24-6477)	816 (119-4228)
% of total T cells	44 (12-78)	34 (16-78)	36 (12-60)	52 (23-73)
% of normal	140 (7-920)	168 (64-494)	134 (7-920)	154 (19-744)

* Median (range)
† Clinical and immunological categories as defined by the US Centers for Disease Control and Prevention (CDC). (25)
‡ Defined as CD45RA+/CD62L+ or CD45RA+/CD27mAb+ CD4+ T-cells
§ Defined as CD45RO+/CD62L- or CD45RO+/CD27mAb- CD4+ T-cells

Naive and memory CD4+ T-cell responses

The median absolute CD4+ T-lymphocyte counts at baseline varied widely among patients (Table 1). According to the CDC guidelines (25) 14 of 71 (20%) patients did not show immunosuppression (CD4+ T-cell count ≥25% of total T-cell count), 28 of 71 (39%) patients showed moderate immunosuppression (CD4+ T-cell count 15-24% of total T-cell count) and 29 of 71 (41%) patients showed severe immunosuppression (CD4+ T-cell count <15% of total T-cell count) at baseline.

The absolute CD4+ T-cell count and the CD4+ T-cell percentage both increased significantly ($p<0.001$) to a median of 939 x 10^6 cells/L (range 10-3520) and 32% (range 1-50%) respectively after 48 weeks of therapy (Figure 2A). In all age groups the increase of total absolute CD4+ T-cell counts was mainly caused by the increase of the naive CD4+ T-cell subpopulation. Memory CD4+ T-

cells did not increase significantly. Therefore the increase of the CD4+ T-cell counts in children was not biphasic as in adults. After an initial increase of the total CD4+ T-cell counts and naive CD4+ T-cell counts, a decrease was seen at week 8 in children younger than 5 years. Outliers could not explain this decrease. After 48 weeks CD4+ T-cell counts reach a plateau consisting of values equivalent to the CD4+ T-cell counts in healthy children. In children younger than 2 years absolute CD4+ T-cell counts even decreased after 48 weeks, reflecting the normal age-related strong decline of CD4+ T-cell counts in children at this age.

The increase of the CD4+ T-cell percentage in the three age groups is depicted in Figure 2B. The ratio of naive and memory CD4+ T-cell counts for the total group increased significantly ($p=0.003$) from 0.67 (range 0.04-4.88) to 1.70 (range 0.09-6.69) after 48 weeks, reflecting the increase mainly consisting of naive CD4+ T-cells.

CD4+ T-cell counts expressed as percentage of normal, increased significantly ($p<0.001$) from 44% (range 0-143%) to 84% (range 1-161%) after 48 weeks. The change of CD4+ T-cell count from baseline was not significantly different between the age groups at any time-point.

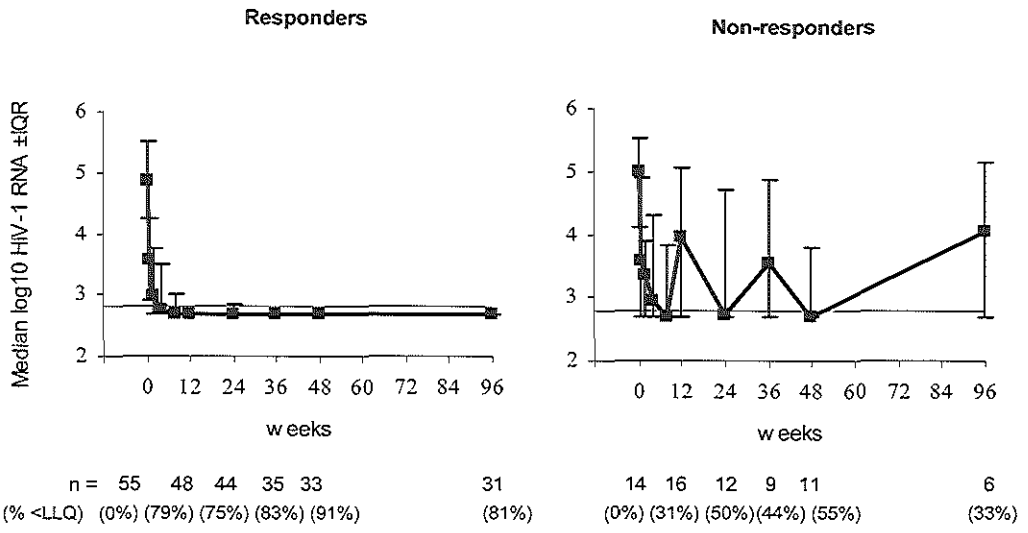

Figure 1: Medians and interquartile ranges (IQR) of HIV-1 RNA with a lower limit of quantification (LLQ) of 500 copies/ml in virologic responders and non-responders after the initiation of HAART. The number of patients which are analysed and the percentage of values below LLQ are indicated.

CD4+ T-cell response and age

High CD4+ T-cell recovery rates have been associated with younger age. (6, 11, 13-16) Indeed a tendency to an inverse correlation was found between the increase of absolute naive CD4+ T-cell counts and the age of the children after 4, 24 (Figure 3A) 36 and 48 weeks of HAART ($r= -0.31$, $p= .03$; $r= -0.34$, $p= .02$; $r= -0.47$, $p= .01$; and $r=-0.33$, $p= .04$ respectively).

We subsequently analysed the increase of naive CD4+ T-cell count relative to the median of the age-specific reference values. (18, 19) Interestingly, when CD4+ T-cell restoration was studied as percentage of normal values, the inverse correlation between the increase of naive CD4+ T-cell count and age was not observed after 4, 24 (Figure 3B) and 36 weeks.

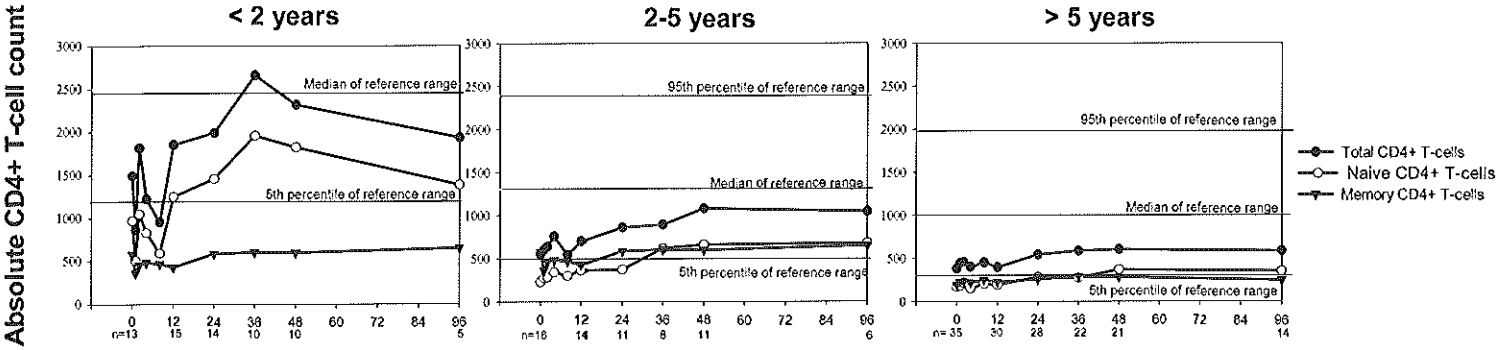

Figure 2A

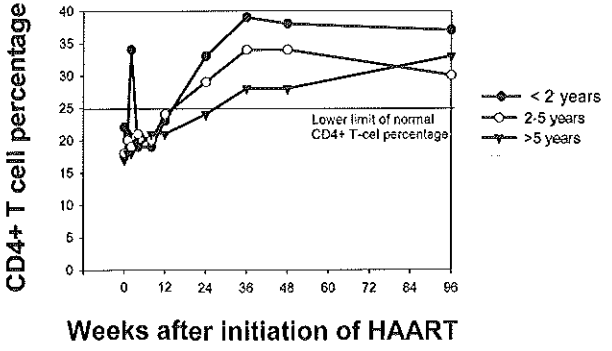

Figure 2B

Figure 2A: Absolute total, naive and memory CD4+ T-cell responses in three age groups (<2 years, 2-5 years and >5 years). The number of patients which are analysed is depicted. **Figure 2B:** The increase of CD4+ T-cell counts as percentages of total T-cell counts in three age groups (<2 years, 2-5 years and >5 years).

Thus, younger children produce more cells in absolute numbers. However, they need to produce more CD4+ T-cells in order to normalise their CD4+ T-cell counts. One may therefore conclude that older children are able to normalise their CD4+ T-cell counts as well as younger children.

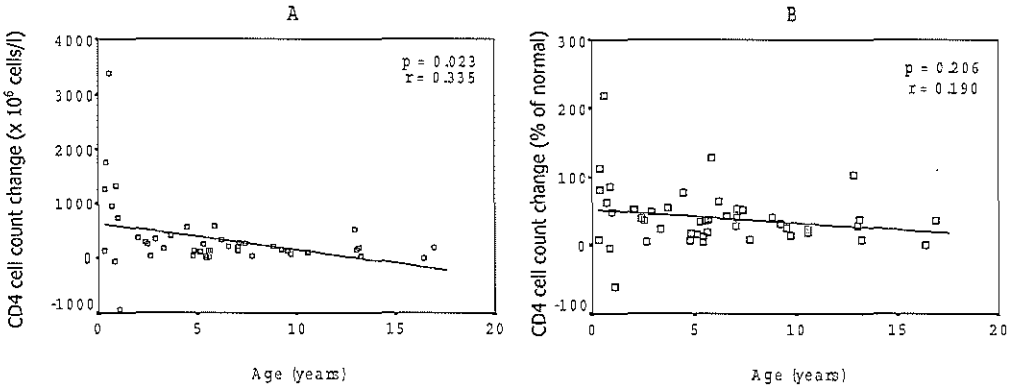

Figure 3A *Relation between the change of absolute naive CD4+ T-cell count and the age of the children after 24 weeks of HAART. The correlation coefficient was calculated by the Spearman rank-correlation method.* ***Figure 3B*** *Relation between the change of naive CD4+ T-cell counts as percentage of normal and the age of the children after 24 weeks of HAART. The correlation coefficient was calculated by the Spearman rank-correlation method.*

CD4+ T-cell response and virologic response

The immunologic reconstitution (absolute CD4+ T-cell counts, CD4+ T-cell counts as percentage of normal, CD4+ T-cell counts as percentage of total T-cell count) was not different at any time-point in virologic responders and virologic non responders. Figure 4A shows the change from baseline of CD4+ T-cell counts as percentage of normal in responders versus non-responders.

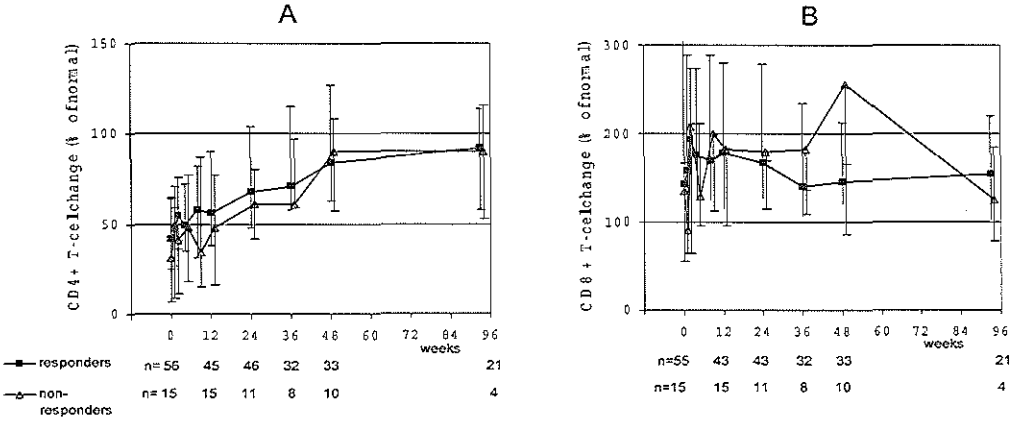

Figure 4A: *Change from baseline of CD4+ T-cell count as percentage of normal in virologic responders versus non-responders.* ***Figure 4B:*** *Change from baseline of CD8+ T-cell count as percentage of normal in virologic responders versus non-responders.*

CD4+ T-cell response and baseline CD4+ T-cell count

Strongly immunosuppressed adults have poor recovery of CD4+ T-cell counts. (17) Therefore, we analysed in our patients the relation between baseline CD4+ T-cell counts and the increase of CD4+ T-cells. We observed a inverse correlation between baseline CD4+ T-cell counts and the change from baseline of CD4+ T-cell counts as percentage of normal after 2, 4, 36, 48 and 96 weeks (r= -0.25, p= .05; r= -0.25, p= .05; r= -0.49, p= .001; r=-0.42, p= .01; and r=-0.41, p= .04 respectively). Thus, children with lower baseline CD4+ T-cell counts showed a larger increase of CD4+ T-cells after the initiation of HAART. Recovery to normal values was seen even in children with very low CD4+ T-cell counts ($<10 \times 10^6$ cells/L) at baseline.

CD8+ T-cell responses

Neither the median absolute CD8+ T-cell counts, nor the CD8+ T-cell percentage of total T-cells nor the CD8+ T-cell percentage of normal did change after the initiation of therapy. No significant difference was found between virologic responders and non-responders in CD8+ T-cell counts as percentage of normal (Figure 4B).

T-cell responses to CD3 plus CD28 mAb stimulation in vitro

To study functional recovery of T cells during HAART, T cell proliferation in vitro was analysed An increase of T-cell responses to CD3 plus CD28 mAb stimulation in vitro from a median (IQR) of 1368 (675-2716 (cpm/10^3 T-cells)) at baseline to a median (IQR) of 2325 (1480-3542 (cpm/10^3 T-cells)) after 48 weeks was observed (p=0.06). Expressed as percentages of the median of 3450 healthy adult donors the T-cell response increased from a median (IQR) of 68% (33-134%) at baseline to 115% (73-175%) at week 48 (p=0.06).

Discussion

In this study the long-term immunologic response to HAART, consisting of one protease inhibitor and two nucleoside analogues, was evaluated in HIV-1-infected children. CD4+ T-cell numbers in HIV-1-infected children on HAART recover more rapidly than CD4+ T-cells in HIV-1-infected adults as has been published by others. (6, 11, 13-16) This good immunological response to HAART has been attributed to the functioning thymus present in young children. Our study is the first in which recovery of CD4+ T-cell counts is related to reference values for lymphocyte subpopulations. In general, absolute CD4+ T-cell counts are used in pediatric studies regarding T-cell repopulation during HAART, since CD4+ T-cells as percentages of total T-cell counts are influenced by the major changes in the number of CD8+ T-cells which are encountered in HIV-1-infected patients (6-8). However, analyses of T cell repopulation in groups of children with different ages are hampered by the fact that CD4+ T-cell counts are highly dependent on the age of the patients. (18, 19) Reference values of younger children (< 2 years) are much higher and have a large range compared to older children (> 2 years). (18, 19) Hence, younger children need to produce larger numbers of CD4+ T-cells to achieve their normal age-related CD4+ T-cell counts. The calculation of CD4+ T-cell counts as percentage of normal absolute values thus results in an independent parameter for the degree of CD4+ T-cell restoration.

Using this method it appears from the data that CD4+ T-cell counts in older children are restored to the same degree relative to their normal values as in younger children. This is in

agreement with the correlation between thymic size and the increase in naive CD4+ T-cell numbers (9, 26) , because younger children, who have a larger thymus, need to produce more naive CD4+ T-cells to recover and maintain normal CD4+ T-cell counts.

Analyses of absolute CD4+ T-cell counts showed that our data are consistent with the previously observed finding that the repopulation of absolute CD4+ T-cell counts is more rapid and more complete in children than in adults. (6, 11, 13-16) Even children with extremely low CD4+ T-cell counts at baseline did reach normal values during the follow-up period.
Reconstitution of the immune system in these children is predominantly caused by the production of naive CD4+ T-cells. The initial increase of memory CD4+ T-cell numbers as observed in adults, is not seen in children. (7, 8)

In addition to the quantitative improvement of the immune system, T-cell function after stimulation with CD3 mAb plus CD28 mAb also improved. Since proliferation of T-cells was expressed as thymidine incorporation per 10^3 T-cells, circulating T-cells had an increased capacity to proliferate after the initiation of HAART. This indicates that there is also functional improvement of T cells during HAART.

A remarkable finding was the absence of differences between virologic responders and virologic non-responders in respect to immunologic reconstitution despite the long term follow-up of 96 weeks. Similar observations have previously also been reported by others. (13, 15, 22, 27) The phenomenon could be explained by the selection of certain viral variants with resistance to protease inhibitors that have in-vitro impaired replicative capacity. (28) Douek et al. also reported an increase of peripheral CD4+ T-cell counts in both virologic responding and non-responding children on antiretroviral therapy. However, they observed that the recovery of thymic function was affected by the degree to which virus suppression was achieved when thymic function was measured by quantifying T-cell receptor rearrangement excision circles in peripheral blood. (29)

Our results indicate that normalisation of CD4+ T-cell count in HIV-1-infected children on HAART is age-independent, suggesting that thymic function allows the children in all age groups to meet their widely different CD4+ T-cell production demands. Remarkably, HAART had a beneficial effect on immune reconstitution regardless of virological success, even when children were in an advanced stage of HIV-1 infection.

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Treatment with highly active antiretroviral therapy in HIV-1 infected children is associated with a sustained effect on growth

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Chapter 12

Abstract

Introduction: Growth failure is a common feature of children with human immunodeficiency virus type 1 (HIV-1) infection. Children who are treated with mono or dual NRTI therapy show a temporary increase in weight gain and linear growth rate. In adults protease-inhibitor-containing antiretroviral therapy is associated with a sustained weight gain and increased body mass index. Experience with protease inhibitors and growth in children is still limited. The data mainly deal with short-term effects on growth. *Objective:* To evaluate the effect of highly active antiretroviral therapy (HAART) on growth in children with human immunodeficiency virus type 1 (HIV-1) infection. *Design & Methods:* We analyzed selected growth parameters, clinical data and laboratory results as part of a prospective, open, uncontrolled, multicenter study to evaluate the clinical, immunologic and virologic response to HAART consisting of indinavir, zidovudine, and lamivudine in children with HIV-1 infection. Height and weight were measured at 0, 12, 24, 36, 48, 60, 72, 84, and 96 weeks after initiation of HAART. Information about the children's growth before enrollment in the study was retrieved from the hospital medical records and or the school doctor or health center. The body mass index (BMI) was calculated. Z-scores were used to express the standard deviation in SD units from the Dutch reference curves for age and gender. Viral loads and CD4+ T cell counts were examined prospectively and related to these growth parameters. Z-scores were also calculated for CD4+ T cell counts to correct for age-related differences. A z-score of 0 represents the P50, which is exactly the age/ sex-appropriate median. A height z-score of −1 indicates that a child's height is 1 standard deviation below the age- and gender-specific median height for the normal population. Virologic responders were defined as those who either reached an undetectable viral load (<500 copies/mL) or had a >1.5 log reduction in viral load compared to baseline at week 12 after the initiation of HAART, which was maintained during the follow-up period.

Results: Patients: Twenty-four patients were included (age 0.4 - 16.3 years at baseline), with a median HIV-1 RNA load of 105,925 copies/ml (=5.03 log), a median CD4+ T-cell count of 0.586 x 10^9/L (median z-score: -2.28 SD), a median height z-score of −1.22, a median weight z-score of -0.74 and a median baseline BMI z-score of −0.32. Eleven patients were naive to antiretroviral therapy and 13 had received prior treatment with NRTI mono therapy. Twenty children used indinavir and 4 children nelfinavir as part of HAART. *Virologic and immunologic responses to HAART:* Seventeen children were virologic responders and seven children virologic nonresponders. In patients naive to NRTIs median baseline viral loads were significantly higher than in pretreated patients (p=0.02). However, at weeks 48 and 96, there was no significant difference between the viral loads of both groups. At baseline, there was no significant difference in CD4+ T cell z-scores between virologic responders and nonresponders or between naive and pretreated patients (p>0.05). During 96 weeks of HAART, the increase of CD4+ T cell z-score was significantly higher in responders than in nonresponders (p=0.008). The increase in CD4+ T cell z-score was not significantly different for naive and pretreated patients (p>0.05). *Height, weight and BMI z-score changes:* We found that there was a trend towards a significantly increased z-score change during 96 weeks of HAART compared to the z-score change before HAART initiation for height (p=0.052) and weight (p=0.056), but not for BMI (p=0.627). *Growth and virologic response to HAART:* When the data were analyzed seperately for virologic responders and nonresponders, virologic responders showed significant increases in height and weight (p=0.035 and p=0.022 respectively). The height

and weight of virologic non-responders did not change significantly. The BMI did not change significantly in responders or in nonresponders. *Growth and immunologic response to HAART:* The increase of weight and BMI z-scores from baseline correlated positively with the CD4+ T cell z-score increase from baseline. It did not correlate with absolute CD4+ T cell count increase. Height z-score increase did not correlate with CD4+ T cell z-score or with absolute CD4+ T cell counts. *Growth and prior NRTI treatment:* The height z-score decrease from week -48 to baseline was significantly larger in naive than in pretreated patients (p=0.007). The weight and BMI z-score change from week –48 to baseline was not significantly different for pretreated and naive patients. From baseline to week 96, the height and weight z-score change increased significantly in naive patients (p=0.033 and p=0.026 respectively) but not in pretreated patients compared to the change from week –48 to baseline. The BMI z-score did not change significantly over 96 weeks of HAART for naive or pretreated patients. *Growth and clinical stage of infection:* The clinical stage of infection according to the CDC classification correlated negatively with the BMI z-score and the weight z-score at baseline but not with the height z-score. Thus, children with the most severe clinical disease had the lowest BMI and weight z-scores at baseline. The BMI z-score increased more in children with more advanced clinical infection at baseline, who had lower BMI at baseline. The clinical stage of infection did not correlate with the change in weight z-score from baseline to week 96 (p>0.05).
Conclusions: HAART has a positive influence effect on the growth of HIV-1-infected children. This effect is sustained for at least 96 weeks. Height and weight are favorably influenced in children in whom HAART leads to a reduction of the viral load of at least 1.5 log or to less than 500 copies/ mL and to an increase in the CD4+ T cell z-score. In contrast to the increase of the BMI in adults on HAART, BMI did not increase in all children effectively treated with HAART. BMI increased more in children with an advanced stage of infection and a poor nutritional status at baseline. Data from pretreated and naive patients were difficult to interpret, since the baseline characteristics of these two groups differed too much.

Introduction

Growth failure is a common feature of children with human immunodeficiency virus type 1 (HIV-1) infection. (1-8) The etiology of this HIV-1-related growth failure is complex. It is not only caused by an inadequate caloric intake, since increases in caloric intake do not seem to increase lean body mass or accelerate the rate of linear growth, but only increase weight and fat mass. (9,10) Previously, a correlation with viral load was recognized. (11) Abnormal function of the thyroid gland, the somatomedine axis, the lipid metabolism, and abnormal resting energy expenditure may also contribute to the diminished growth. (12,13) Growth seems to be one of the most sensitive indicators of disease progression in children with AIDS. The absence of growth indicates a poor prognosis, also in children who are treated with antiretroviral regimes.(14-19) Poor growth commonly precedes a decline in CD4+ T-cell count and the subsequent development of opportunistic infections. (20,21) Several studies have shown that weight gain may be an important indicator of antiretroviral therapeutic efficacy. (22,23) Children who are treated with mono or dual therapy containing zidovudine, didanosine, or zalcitabine show a temporary increase in weight gain and linear growth rate. (24-26) In adults protease-inhibitor-containing antiretroviral therapy is associated with a sustained weight gain and increased body mass index. (27) Experience with protease

inhibitors and growth in children is still limited; it mainly consists of short-term effects on growth. (28-31) The objective of this study was to evaluate the effect of highly active antiretroviral therapy (HAART) consisting of one protease inhibitor and two nucleoside analogue reverse transcriptase inhibitors (NRTIs) on the long-term growth profile of HIV-1-infected children.

Methods

Selected growth parameters, clinical data and laboratory results were analyzed as part of a prospective, open, uncontrolled, multicenter study to evaluate the clinical, immunologic and virologic response to HAART consisting of indinavir, zidovudine, and lamivudine in children with HIV-1 infection. (32) The study protocol was approved by the medical ethical committees of all the participating centers. Written informed consent was obtained from parents or legal guardians. Children > 3 months of age with HIV-1 infection and one of the following 2 items: a decreased CD4+ T-cell count (<1 year: <1,750/mm^3, 1-2 years: <1,000/mm^3, 3-6 years: <750/mm^3, >6 years: <500/mm^3) or a HIV-1 viral load > 5,000 copies/mL were included. Growth data prior to HAART initiation also needed to be available in order to be included. Height and weight measurements that were and blood samples for virologic and immunologic parameters were obtained twice within a month before the start of HAART, and after 12, 24, 36, 48, 60, 72, 84, and 96 weeks of treatment. Height and weight measurements were obtained by a single investigator using the same scales and the same metal measuring rod every time. Information about the children's growth before enrollment in the study was retrieved from the hospital medical records and or the school doctor or health center. Height and weight measurements obtained closest to 24 and 48 weeks before HAART initiation were entered into the database.

Virologic responders were defined as those who either reached an undetectable viral load (<500 copies/mL) or had a >1.5 log reduction in viral load compared to baseline at week 12 after the initiation of HAART, which was maintained during the follow-up period.

The BMI was calculated from height and weight values as defined by BMI= weight (kg)/(height (m))^2. BMI provides an indication of the nutritional status of the patients. Compared to the two other measurements for weight-for-height, i.e. kg/m and kg/m^3, BMI has the desired lower correlation with height and higher correlation with weight and skinfold thickness. (33) Z-scores were used to express the deviation in SD units from the Dutch reference curves for age and gender. (34,35) The z-scores for weight, height, and BMI were calculated by means of the SDS software program (version 2.0, Erasmus University, Rotterdam, 1996). Reference growth curves were not available for most countries from which the patients originated. Therefore, Dutch reference growth curves were used. Since we only discuss change in z-scores over time and not absolute z-scores, this does not present any problems. Z-scores were also calculated for CD4+ T cell counts to correct for age-related differences. (36) A z-score of 0 represents the P50, which is exactly the age/ sex-appropriate median. A height z-score of –1 indicates that a child's height is 1 standard deviation below the age- and gender-specific median height for the normal population.

The viral load was measured using the Roche Amplicor HIV-1 Monitor test. (37) CD4+ T cell counts were obtained by standard flow cytometric methods. Statistical calculations were performed with the SPSS statistical analysis software program (version 10.0). The relations between growth parameters, CD4+ T lymphocyte counts, CD4+ z-scores, and viral loads were analyzed using the Wilcoxon signed-rank test, Mann-Whitney U test, and Spearman rank correlation test. All p-values are two-tailed.

Results

Population characteristics

Twenty-four HIV-1-infected children were included. They all completed at least 96 weeks of HAART. Baseline characteristics of these 24 children are presented in table 1. The median age of the children was 5.2 years (range: 4.6 months- 16.3 years). Eleven patients were not previously treated and 13 had received prior treatment with NRTIs, mostly zidovudine mono therapy for an average of 30 months (range: 8.4 – 106.2 months). Pretreated children were significantly older at baseline than NRTI-naive children (p=0.002), with a median age of 7.4 years and 2.0 years, respectively. Twenty children received HAART containing indinavir and 2 NRTIs and 4 children received HAART containing nelfinavir and 2 NRTI's. In 5 children indinavir was changed to nelfinavir and in 1 child to indinavir and ritonavir and later to nevirapine because of a viral load rebound after a median duration of 48 weeks. In 2 other children indinavir was changed to nelfinavir because of long-term side effects.

Table 1: Baseline Characteristics of Study Patients (n=24)

Median age in years (range)	5.2 (0.4- 16.3)
Sex (male/female), n=	11/13
Race/ethnicity, n=	
Non white	19
White	5
Route of acquisition, n=	
Vertical	16
Blood products	4
Unknown	4
CDC classification, n=*	
N 1/ 2	2/ 3
A 1/ 2/ 3	2/ 3/ 4
B 1/ 2	1/ 4
C 2/ 3	2/ 3
No prior treatment, n=	11
Prior treatment with, n=	
zidovudine	11
zidovudine/ zalcitabine	2
Log_{10} copies of HIV RNA/ml plasma, median (range)	*5.03 (3.42 to 5.87)*
CD4+ cell count in 10^9/L, median (range)	*0.586 (0.010 to 3.580)*
CD4+ z-score in SD, median (range)	*-2.28 (-13.75 to 0.26)*
Z-score in SD, median (range)	
Height	-1.22 (-3.84 to 1.25)
Weight	-0.74 (-3.13 to 2.26)
BMI	-0.32 (-3.88 to 2.03)

*Clinical and immunologic categories as defined by the US Centers for Disease Control and Prevention (CDC). (38)

Virologic response

The median baseline plasma viral load of 5.03 log_{10} copies/mL (range, 2.43-5.88) decreased to less than 2.70 log_{10} copies/ml by week 48 and remained below the level of detection until at least week 96. In patients naive to NRTIs (n=11) median baseline viral loads were significantly higher than in pretreated patients (n=13) (p=0.02). However, at weeks 48 and 96, there was no significant

difference between the viral loads of both groups (Table 2). Seventeen children were virologic responders and seven children virologic nonresponders.

Table 2: Viral load at baseline, 48 weeks and 96 weeks after HAART initiation in Log copies per ml, median (25^{th} and 75^{th} percentile)

Patients	Baseline	Week 48	Week 96
All	5.03 (4.29-5.60)	<2.70 (<2.70- <2.70)	<2.70 (<2.70- 3.29)
Naive	5.41 (5.16- 5.85)*	<2.70 (<2.70- <2.70)	<2.70 (<2.70- 2.85)
Pretreated	4.47 (4.09- 4.93)*	<2.70 (<2.70- 2.76)	<2.70 (<2.70- 3.47)
Responders	5.16 (4.32- 5.66)	<2.70 (<2.70- <2.70)^	<2.70 (<2.70- <2.70) #
Non-responders	4.94 (4.24- 5.29)	<2.70 (<2.70- 3.80) ^	3.29 (3.08- 4.34) #

*significant difference, p= 0.02, ^ significant difference, p=0.024, # significant difference, p<0.0001

Immunologic response

Absolute CD4+ T cell counts per age group are shown in figure 1a. The median baseline CD4+ T cell z-score was –2.28 SD (range, -13.75 to 0.26). At baseline, there was no significant difference between virologic responders and nonresponders or between naive and pretreated patients (p>0.05). During 96 weeks of HAART, the increase of CD4+ T cell z-score was significantly higher in responders than in nonresponders (p=0.008) (Figure 1b). The increase in CD4+ T cell z-score was not significantly different for naive and pretreated patients (p>0.05).

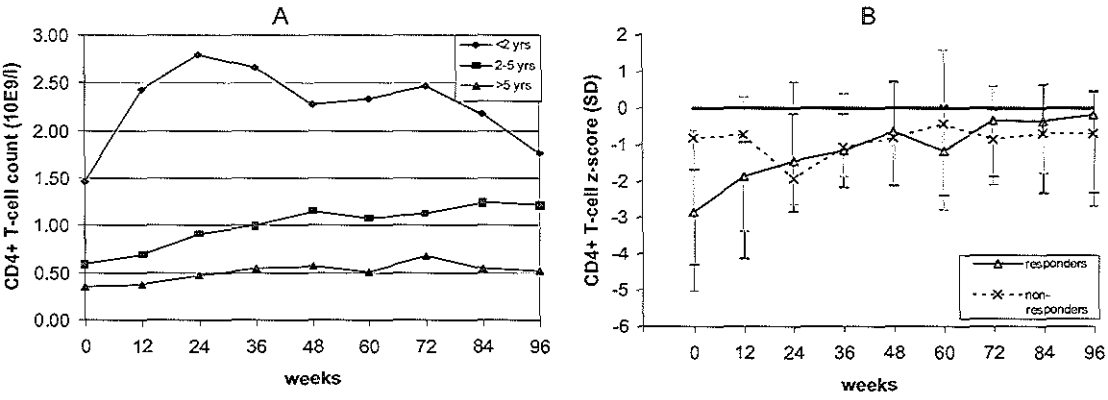

Figure 1a: *CD4+ T-cell count change in different age categories during 96 weeks of HAART.* ***Figure 1b:*** *CD4+ T-cell z-score change in responders and non-responders during 96 weeks of HAART.*

Height, weight and BMI z-score changes

Using data from all patients regardless of virologic response and prior treatment, the effects of HAART on growth were determined. We found that the height z-score decreased in the 48 weeks before HAART initiation with a median (range) of –0.088 SD (-1.88 to 1.17) to a median of –1.22 SD at baseline. From baseline to 96 weeks after the initiation of HAART the height z-score increased with a median (range) of 0.20 SD (–0.72 to 1.29) to a median of –0.95 SD. The weight z-score decreased from week –48 to baseline with a median (range) of –0.041 SD (-4.10 to 1.96) to a

median of –0.74. From baseline to week 96 the weight z-score increased with a median of 0.34 SD (-0.83 to 2.13) to a median of –0.60. The BMI z-score change from week –48 to baseline decreased with a median (range) of –0.12 SD (–4.10 to 1.96) to a median of –0.32 SD. From baseline to week 96 the median (range) BMI z-score change was 0.28 SD (-1.75 to 3.60) to a median of 0.19 SD. We found that there was a trend towards a significantly increased z-score change during 96 weeks of HAART compared to the z-score change before HAART initiation for height (p=0.052) and weight (p=0.056), but not for BMI (p=0.627).

Growth and virologic response to HAART

The median change in height z-score decreased in the 48 weeks before HAART initiation. Children with a higher viral load at baseline showed a larger decrease (p<0.0001) of the height z-score in the 48 weeks before HAART initiation than children with a lower viral load. From baseline to week 96 height z-score and weight z-score changes increased significantly in virologic responders, but not in nonresponders compared to the change from week –48 to baseline (Figures 2a-d).

BMI z-score did not increase significantly over 48 or 96 weeks of HAART in responders or in non-responders. (Figure 2e and 2f) However, the BMI z-score change increased significantly more in virologic responders than in nonresponders to HAART (p=0.024 after 96 weeks).

Growth and immunologic response to HAART

During 96 weeks of HAART, the change in height z-scores from baseline did not correlate with the change in CD4+ T cell z-scores or absolute CD4+ T cell counts.

The change in weight z-scores from baseline correlated positively with the change in CD4+ T cell z-scores at week 24 (r=0.693, p<0.0001), 36 (r=0.543, p=0.007), 48 (r=0.628, p=0.001), 60 (r=540, p=0.009), 84 (r=0.496, p=0.019) and 96 (r=0.408, p=0.048). The change in weight z-scores only correlated with the change in absolute CD4+ T cell counts at week 36 (r=0.439, p=0.032).

The change in BMI z-scores correlated positively with the change in CD4+ T cell z-scores at al time points from week 12 onwards except at week 84: week 12 (r=0.476, p=0.019), 24 (r=0.671, p<0.0001), 36 (r=0.508, p=0.011), 48 (r=0.592, p=0.003), 60 (r=0.443, p=0.039), 72 (r=0.520, p=0.013), 84 (r=0.369, p=0.091) and 96 (r=0.501, p=0.013). The change in BMI z-scores did not correlate with change in absolute CD4+ T cell counts.

Growth and prior NRTI treatment

The height z-score decrease from week -48 to baseline was significantly larger in naive (-0.41 SD) than in pretreated (-0.04 SD) patients (p=0.007). From baseline to week 96, the height z-score change increased significantly in naive patients (p=0.033) but not in pretreated patients compared to the change from week –48 to baseline.

The weight and BMI z-score change from week –48 to baseline was not significantly different for pretreated and naive patients. The weight z-score change increased significantly over 96 weeks of HAART for naive patients (p=0.026) but not for pretreated patients.

The BMI z-score did not change significantly over 96 weeks of HAART for naive or pretreated patients. There was no significant difference between naive and pretreated patients either.

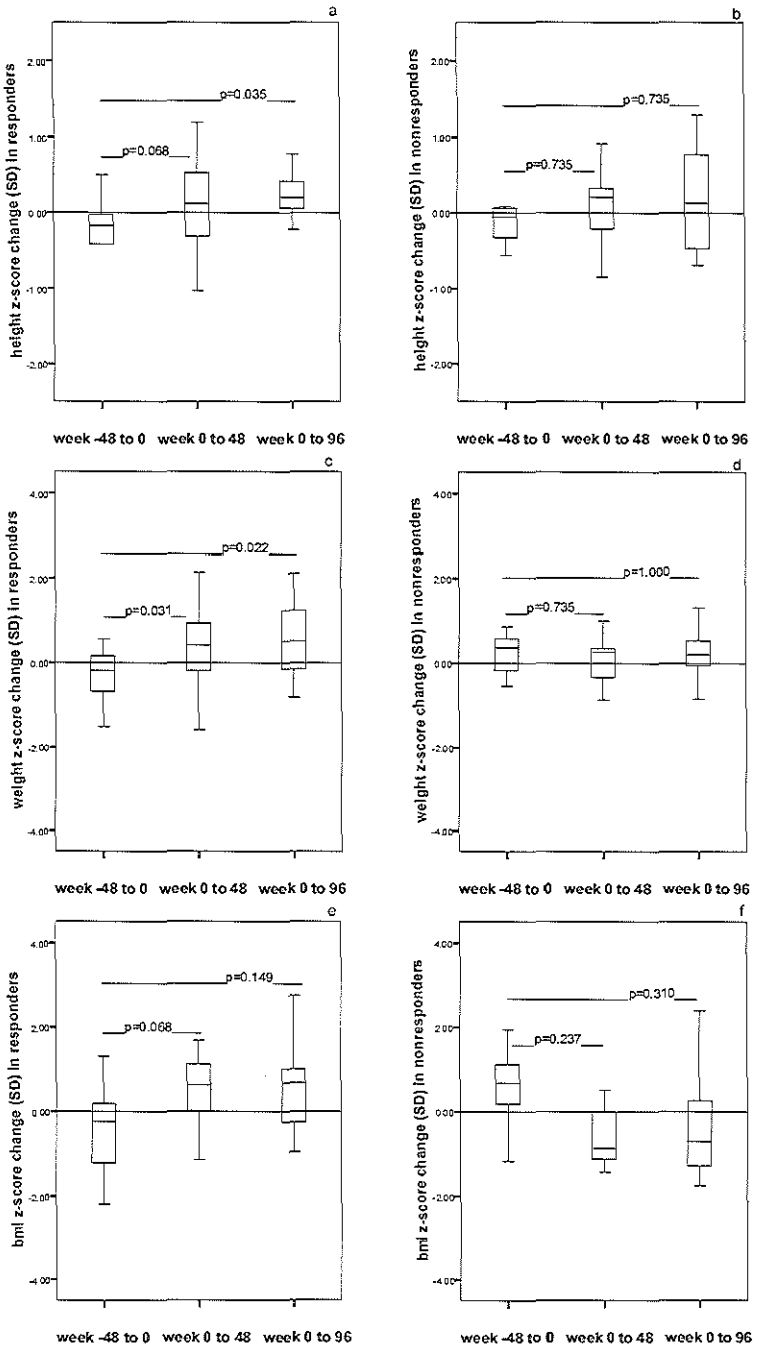

Figure 2: *Growth before and during HAART in (a, c and e) responders and (b, d and f) non-responders. Growth and clinical stage of infection*

The clinical stage of infection according to the CDC classification (38) correlated negatively with the BMI z-score at baseline. Thus, children with the most severe clinical disease had the lowest BMI z-scores at baseline. The BMI z-score increased more in children with more advanced clinical infection at baseline, who had lower BMI at baseline.

The clinical stage of infection at baseline also correlated negatively with weight z-score but not with the height z-score. Children with advanced clinical infection had lower weight for their age and gender at baseline. The clinical stage of infection did not correlate with the change in weight z-score from baseline to week 96 ($p>0.05$).

Discussion

The results of this study indicate that children with HIV-1 infection show a trend to an increase in height and weight ($p=0.052$, $p= 0,056$ respectively) after the initiation of HAART. However, when the children were divided into virologic responder and nonresponder groups, the responders showed significant increases in height and weight, whereas nonresponders did not. The BMI did not change significantly in responders or in non-responders, although it increased more in responders than in nonresponders. Increasing CD4+ T-cell counts favorably influenced weight and BMI. Clinical stage of infection was also correlated with the increase of BMI from baseline to week 96 of HAART. BMI z-scores increased more in children with an advanced clinical stage of infection at initiation of HAART. The energy expenditure previously needed to combat infection was possibly used for catch-up growth. CD4+ T cell z-scores then relate to BMI and weight only indirectly.

In HIV-1 infected adults receiving HAART, BMI increased significantly more in responders than in nonresponders, but BMI also increased significantly overall, in responders and in nonresponders. BMI increase consisted mainly of increased fat mass. Lean body mass did not increase significantly. (27) We are not aware of any previously published studies on the influence of HAART on BMI in HIV-1-infected children. However, in a recent study in HIV-1-infected children protease inhibitors were found to cause a significant increase in weight-for-height and a dramatic improvement in lean body mass over a short interval and had no immediate influence on fat mass, although there was a trend toward increased fat mass, with longer follow-up time. (28) This suggests that in the children whose BMI increased in our study, namely the children with an advance stage of clinical infection and responders to HAART with a high increase in CD4+ T cell count, lean body mass also increased. The difference of the effect of HAART on body composition between children and adults could be attributed to the fact that children are still growing so their metabolism is different, but more research is needed into this.

Height did not increase significantly in responders until week 96 while weight already increased significantly at week 48. This is a normal reaction to the correction of a growth-retarding disorder: catch-up growth first affects weight followed by height. (39)

At the initiation of HAART viral loads correlated negatively with height z-score change in the previous year and the clinical stage of infection correlated negatively with BMI z-score and weight z-score. Children with high viral loads and severe clinical infection also had poor grow parameters. These are all associated with a poor prognosis. Although the number or percentage of CD4+ T cells has a larger influence on prognosis, increasing growth rates also contribute to a better prognosis. (16,18,19,22,23)

Height and weight z-score change over 96 weeks compared to z-score change in the 48 weeks before HAART initiation increased more in responders than in non-responders. It also

increased more in naïve patients than in pretreated patients. Although this could be interpreted as a confounder, it is more likely to be the result of previous antiretroviral therapy. Pretreated patients seem to have already reached a higher height z-score change during the 48 weeks before HAART initiation. This may have been caused by the treatment with NRTIs. Weight z-score change in the 48 weeks before HAART initiation was not significantly different between naive and pretreated patients. We concluded from the observation that weights increased in naive patients and not in pretreated patients. However, a difference was observed in baseline viral load and age: pretreated patients were significantly older and had a significantly lower viral load. This complicates the comparison between these two groups of patients.

Similar results in height and weight gain as observed by our group were reported in three other studies on the effects of HAART in children. (28,30,31) However, the follow-up time of these studies was only 24 weeks. A positive influence on growth has been observed as a result of NRTI mono therapy or dual therapy during this same period. (14,22,24,40-46) The effect on growth of mono therapy cannot be sustained after 24 weeks of treatment. There is no data, which support that dual therapy has a sustained effect on growth beyond 24 weeks. The current study shows that the positive effects of HAART on growth can be sustained for at least 96 weeks. Therefore, the effect on growth lasts longer in patients receiving HAART than in patients receiving mono or duo reverse transcriptase inhibitor therapy. There seems to be a relation between the time that an antiretroviral therapy is successful in the suppression of viral load and the time that the positive effect on growth by this therapy can be maintained. Ogino et al. (22) also raised this point when they described the effect of zidovudine resistance on growth.

Dreimane et al. (29) retrospectively reviewed 27 HIV-1-infected children receiving HAART for a mean follow-up time of 20 months. They also found increased z-scores for height and height-velocity, but not for weight. This difference in findings may be attributed to the low number of responders to HAART in their study (10 out of 27) compared to the high number of responders (17 out of 24) in our study. It could also be attributed to the fact that they used another protease inhibitor. Unfortunately, they did not mention which protease inhibitor was used in their patients. Miller et al. (28) found that ritonavir had a weaker effect on weight with a stronger effect on height compared to indinavir and nelfinavir.

Conclusion

HAART has a positive effect on height and weight in children with HIV-1 infection. This effect is sustained for at least 96 weeks and is associated with the successful application of HAART, resulting in long-term viral load reduction of at least 1.5 log copies/mL or viral load suppression below 500 copies/mL and an increase of CD4+ T-cell counts. The sustained effects of HAART on growth may positively influence the child's quality of life and will predictably contribute to a better prognosis. The mechanism of increased growth during antiretroviral therapy is yet unknown. Contrary to the BMI increase in adults treated with HAART, BMI in children does not increase in all patients successfully treated with HAART, but only in those with a low BMI at initiation of HAART. More research is needed to investigate whether those factors causing growth failure in children with HIV-1 infection – caloric intake, thyroid and growth hormones, lipid metabolism and resting energy expenditure, also play a role in the recovery of growth parameters during HAART.

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Endocrinologic and immunologic factors associated with recovery of growth in HIV-1 infected children treated with protease inhibitors

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Chapter 13

Abstract

Introduction: Growth failure is one of the most common presenting signs in children with human immunodeficiency virus (HIV) disease and forms a sensitive indicator of disease progression in children with AIDS. Highly active antiretroviral therapy (HAART) is not only associated with a significant decrease in viral load and a subsequent rise in CD4+ T-cell counts in HIV-1 infected children, but also with increased height and weight. However, the underlying mechanisms of catch-up growth during HAART are yet unknown. *Methods*: Height and weight measurements, blood sample analyses for HIV-1 RNA and peripheral blood CD4+ T-cell counts were obtained twice within a month before the start of HAART, and after 12, 24, 36, and 48 weeks of treatment. Serum levels of IGF-1, IGFBP-3, cortisol, free thyroxine and TNF-α were measured before the start of the therapy and after 24 weeks. In addition, serum levels of IGF-I and IGFBP-3 were determined after 48 weeks. *Results*: Twenty-seven HIV-1 infected children with a median age of 5.5 years (range: 0.3-14.9 years) were included. Overall, no significant changes in height and BMI z-scores were observed. However, in a subgroup of patients with a change in height or BMI z-scores above the 75th percentile after 48 weeks, a significant change in height and BMI z-scores was observed. The median baseline plasma viral load of 68,800 copies/ml decreased to less than the detection limit of 500 copies/ml in 80% of the children after 48 weeks. TNF-α levels were elevated (44 pg/ml) at baseline and decreased significantly to 37 pg/ml after 24 weeks. At baseline elevated TNF-α levels were observed in 78%, which decreased to 55% after 24 weeks. Baseline levels of free T4 and cortisol were within the normal range and did not change during therapy. Baseline serum levels of IGF-1 and IGFBP-3 were within the normal range, but IGF-1 levels tended to be lower than IGFBP-3 levels. Both levels increased significantly after the initiation of therapy. IGFBP-3 levels decreased after 48 weeks whereas IGF-1 levels stabilized. The increase in the levels of IGF-1 levels was significantly higher in the subgroup of children in whom body mass index and height z-scores increased. *Conclusion:* Hypothyroidism and adrenal axis abnormalities are not associated with restoration of growth after the initiation of antiretroviral therapy in HIV-1 infected children. The combination of relatively high serum levels of IGFBP-3 and relatively lower IGF-1 levels suggests the presence of a GH resistant state. During treatment with a PI-containing regimen, decreased levels of IGFBP-3 and stabilization of IGF-1 levels after a significant initial increase suggest restoration of normal sensitivity to GH and a recovery to an anabolic condition.

Introduction

Growth failure is one of the most common signs of pediatric human immunodeficiency virus (HIV) disease and is one of the most sensitive indicators of disease progression in children with AIDS. Increased morbidity and mortality has been observed in HIV-1 infected children with poor growth. (1-15) The introduction of highly active antiretroviral therapy (HAART) consisting of a protease inhibitor and two nucleoside analogues has resulted in significant improvements in the morbidity and mortality in both HIV-1 infected adults and children. (16-19)In HIV-1 infected children administration of HAART is not only associated with a significant decrease in HIV-1 viral load and an increase in the number of CD4+ T-cells, but also with height and weight gain. (20-25) Since the rate of growth is closely related to the level of HIV-1 replication in HIV-1 infected children, recovery of growth can be partly attributed to the decreases of viral load in children treated with HAART. (15, 26) However, the

mechanism of increased growth during HAART are not yet understood. It has not been investigated whether the influence of therapy on factors causing growth failure in HIV-1 infected children, such as inadequate caloric intake, endocrine disorders (thyroid dysfunction and growth hormone (GH) deficiency), chronic or recurrent infections, malabsorption, abnormal cytokine production, altered fat metabolism and increased total energy expenditure, may play a role in the recovery of growth during HAART.

Hormones that influence growth postnatally include GH and insulin-like growth factor I (IGF-I) as well as thyroid hormone and glucocorticoids. Excessive levels of glucocorticoids or very low levels of thyroid hormone result in growth retardation during childhood. These hormones play a permissive role in promoting growth stimulated by GH and IGF-I. IGF-I mediates the growth promoting actions of GH, a pituitary hormone with highly fluctuating blood levels due to a pulsatile release. (27) The blood concentration of IGF-I is more stable due to the binding to carrier proteins. In serum and other biological fluids, the IGFs are complexed to specific, structurally homologous binding proteins (IGFBPs). IGFBP-3 is the major IGFBP in postnatal serum. (28) Tumor necrosis factor -α (TNF-α) is a pro-inflammatory and immunomodulatory cytokine with an increased production rate in the presence of infectious diseases and malignancies. TNF-α also strongly inhibits lipoprotein lipase and adipocyte gene expression. Chronically high levels of TNF-a have been associated with the development of cachexia.

The aim of this study was to evaluate changes in endocrinological and immunological factors and to analyze their association with recovery of growth during HAART. Height, weight and serum levels of cortisol, the free fraction of circulating thyroxine (free T4), IGF-1, IGFBP-3, TNF-α, HIV-1 RNA and CD4+ T-cells were determined at baseline and 24 and/or 48 weeks after the initiation of HAART.

Methods

Selected growth parameters, clinical data and laboratory results were analyzed as part of a prospective, open, uncontrolled, multicenter study to evaluate the clinical, immunologic and virologic response to HAART consisting of indinavir, zidovudine, and lamivudine in children with HIV-1 infection. (20) The study protocol was approved by the medical ethical committees of all participating centers. Written informed consent was obtained from parents or legal guardians. HIV-1 infected children with an HIV-1 viral load of more than 5000 copies/ml and/or a CD4 cell count below the age-specific reference values were included. (29) Height and weight measurements and blood samples for HIV-1 RNA and CD4+ T-cell count were obtained twice within a month before the start of HAART, and after 12, 24, 36, and 48 weeks of treatment. Blood samples for IGF-1, IGFBP-3, cortisol, free T4 and TNF-α were obtained before start of the therapy and after 24 weeks. Serum IGF-I and IGFBP-3 levels were also determined after 48 weeks. Serum samples were stored at -70°C until assayed.

Statistics

Statistical calculations were performed with the SPSS statistical analysis software program (version 9.0). The BMI was calculated from height and weight values as defined by BMI= weight (kg)/height^2 (m^2). BMI provides an indication of the nutritional status of the patients. Compared to the two other

measurements for weight-for-height, i.e. kg/m and kg/m3, BMI has the desired lower correlation with height and higher correlation with weight and skinfold thickness. (30) Z-scores were used to express the deviation in SD units from the Dutch reference curves for age and gender. (31, 32) Z-scores represent the number of standard deviations above or below the median of the parameter for that sex and age. The z-scores for weight, height, BMI, IGF-1 and IGFBP-3 were calculated by means of the SDS software program (version 2.0, Erasmus University, Rotterdam, 1996).

Because absolute CD4+ T-cell counts are highly dependent on the age of the patients relative CD4+ T-cell counts in relation to the median of the age-specific reference values (29) were calculated by dividing the individual value at the different time-points by the median of the reference value at the different time-points. Results are expressed as a percentage of normal. Virologic responders were defined as those who reached an undetectable viral load (<500 copies/mL) at week 12 after the initiation of HAART, which was maintained during the follow-up period.
The relations between growth parameters, CD4+ T-cell counts, TNF-α, IGF-1, IGFBP-3, cortisol, free T4 thyroid hormone and HIV-1 RNA concentrations were analyzed using the Wilcoxon signed-rank test, Mann-Whitney U test, and Spearman rank correlation test. All p-values are two-tailed.

Laboratory procedures

HIV-1 RNA was measured using the Amplicor HIV-1 monitor test version 1.5 Roche Diagnostic Systems. CD4+ T-cell counts were obtained by standard flow cytometric methods. Total serum IGF-I concentrations were measured by radioimmunoassay (SM-C-RIA-CT, Biosource) The intra- and interassay coefficients of variation were 4,1-6,1% and 9,6%, and RIA sensitivity was 0,25 ng/ml.
Serum IGFBP-3 was determined by a two site immunoradiometric assay (IGFBP-3 IRMA DSL-6600, Diagnostic Systems Laboratories inc.) The intra-assay coefficient of variation was 1,8-3,9%, the interassay coefficient of variation was 0,5-1,9%, and the low detection limit was 0,5 ng/ml. Serum cortisol was evaluated by a solid-phase chemiluminescent enzyme immunoassay (Immulite Cortisol). The intra- and interassay coefficients of variation were 6,8-9,0% and 9,9-10,3%. The sensitivity was approximately 0,2 µg (5,5 nmol/l). Serum free fraction of circulating thyroxine (T4) was measured by a direct labeled antibody competitive immunoassay technique (Amerlite MAB FT4 assay). The inter- and intra-assay coefficients of variation were 4,2-9,0% and 3,5-7,6%, and the low detection limit was 0,7 pmol/l. Serum TNF-α was quantified by a solid phase Enzyme Amplified Sensitivity Immunoassay (TNF-α EASIA, Biosource). The intra- and interassay coefficients of variation were 3,7-5,2%. The minimum detectable concentration was 3 pg/ml.

Results

Patients

Twenty-seven HIV-1 infected children were included. They all completed at least 48 weeks of HAART. Baseline characteristics of these 27 children are presented in table 1. The median age of these children was 5.5 years (range: 0.3-14.9 years). Fifteen children had received prior treatment with NRTI's, mostly with zidovudine for an average of 22.5 months (range 1-43 months). Pretreated children were significantly older at baseline than NRTI-naive children (p=0.02) with a median age of 7.4 years and 4 years respectively. Twenty-four children received HAART containing indinavir and 2 NRTIS' s and 3 children received HAART containing nelfinavir and 2 NRTI's. In 4 children indinavir

was changed to nelfinavir and in 1 child to indinavir and ritonavir, because of a viral load rebound after a median duration of 24 weeks.

Table 1: Baseline characteristics of study patients (n=27)		
Median age in years (range)	5.5 (0.3-14.9)	
Sex: male/female	16/11	
Race/ethnicity		
Non white	23	(85%)
White	4	(15%)
Route of acquisition		
Perinatal	19	(70%)
Blood products	4	(!5%)
Unknown	4	(15%)
CDC Classification (33)		
N 1/2/3	1/4/1	
A 1/2/3	3/6/2	
B 1/2/3	0/3/1	
C 1/2/3	0/3/3	
Prior treatment		
No	12	(44%)
ZDV	13	(48%)
2 NRTI's	2	(7%)
Treatment		
IDV/ZDV/3TC	24	(89%)
NFV/ZDV/3TC	2	(7%)
NFV/d4T/ddI	1	(4%)
Median HIV-1 RNA copies/ml (range)	68,800 (3,170 - 820,000)	
Median CD4+ T-cell count in 10^9/l (range)	440 (10-3580)	
Median CD4+ T-cell count as % of normal (range)	44% (0-143)	

Height and BMI z-score changes

In the total group no significant changes in height and BMI z-scores were observed. Median (range) height z-scores were -1.3 SD (-3.7 to 1.61) at baseline, -1.3 SD (-3.5 to 1.18) after 24 weeks and -1 SD (-3.3 to 0.8) after 48 weeks of therapy. Median (range) BMI z-scores were -0.1 SD (-2.4 to 1.7) at baseline, 0.19 SD (-2.9 to 1.8) after 24 weeks and 0.13 SD (-2.3 to 1.4) after 48 weeks. However in a subgroup of children with a change in height z-scores above the 75th percentile after 48 weeks (n = 12), i.e the children with the highest growth velocity from baseline to week 48, the median (range) change in height z-scores was 0.46 SD (0.05 to 1.99) after 48 weeks (p=0.002). The median (range) change in BMI z-scores after 48 weeks in the children with a change in BMI z-score above the 75th percentile after 48 weeks (n = 6) was 1.16 SD (0.91 to 1.25) (p=0.03).

Virological response rate

The median baseline plasma viral load of 68,800 copies/ml (range: 3,170 - 820,000) decreased to less than the detection limit of 500 copies/ml in 74% of the patients after 24 weeks and in 80% after 48 weeks. Twenty children were virologic responders (< 500 copies/ml at week 12) and seven children virologic non-responders.

Immunological response

The median absolute CD4+ T-cell count (range) of 440 x 10^9/l (10-3580) increased to 630 x 10^9/l (10-4380) after 24 weeks and to 840 x 10^9/l (10-3520) after 48 weeks. CD4+ T-cell counts expressed as percentage of normal (range) of 44% (0%-143%) increased to 66% (1%-99%) after 24 weeks and to 72% (1%-161%) after 48 weeks.

Cortisol and free T4

Both cortisol and free T4 levels did not change significantly during HAART. Before the initiation of therapy, and after 24 weeks of therapy they were within the normal range (cortisol morning samples, 200 – 600 nmol/l and free T4 11 – 25 pmol/l). The median (range) baseline level of cortisol was 278 nmol/l (123 – 765 nmol/l). After 24 weeks of therapy the median (range) cortisol level was 350 nmol/l (136 – 633 nmol/l) (p=0.741). All cortisol samples were drawn in the morning.
The median (range) baseline of free T4 was 18,5 (12.8 – 24.2 pmol/l). After 24 weeks of therapy median (range) free T4 was 18.9 (13.2 – 23.5 pmol/l) (p=0.791).

TNF-α

At baseline elevated TNF-α levels (>30 pg/ml) were observed in 21 of the 27 patients (78%). The median baseline TNF-α concentration of 44 pg/ml (range, 7-164 pg/ml) decreased to 37 pg/ml (range, 6-79 pg/ml) after 24 weeks (p=0,025). After 24 weeks elevated TNF-α levels (>30 pg/ml) were detected in 15 of the 27 patients (55%). During therapy TNF-α levels decreased in 18 children. TNF-α levels increased in 9 children. When children who had an acute infection at one or both time points (n=9) were excluded from analyses, TNF-α levels decreased from a median of 48.5 pg/ml (range 13-164) at baseline to a median of 27 pg/ml (range 6–79 pg/ml) (p=0.01). In the group of children with an acute infection at one or both time points, no significant decrease was seen.
The decrease in TNF-α levels during therapy was not different between children with no or moderate immunodeficiency and children with severe immunodeficiency at baseline. Neither did we observe a difference between virological responders and non-responders in the decrease in TNF-α levels during therapy.

IGF-I and IGFBP-3

IGF-1 increased significantly during therapy from a median (range) of 13.2 nmol/l (2.2-99) at baseline to 22.8 (3.4-113) nmol/l after 24 weeks (p<0.001). After 48 weeks median IGF-1 levels stabilized with a median (range) of 22.5 nmol/l (7.7-64.5). Corrected for age and sex, z- scores of IGF-I also increased significantly during therapy from a median of –0.65 SD (range, -5,00 to 3.20) at baseline to +1.50 SD (range, -4.01 to 3.84) after 24 weeks of therapy (p=0.001) and 0,60 SD (range, -2,50 to 4,20) after 48 weeks of therapy.

IGFBP3 increased significantly during therapy from a median (range) of 92 nmol/l (38-198) at baseline to 123 nmol/l (36-244) after 24 weeks (p=0.001). After 48 weeks median IGFBP-3 levels decreased to a median (range) of 95 nmol/l (28-143). Corrected for age and sex, SD scores of IGFBP-3 also increased significantly during therapy from a median of 1.4 SD (range, -2,50 to 2.60) at baseline to 1.90 SD (range, -3.3 to 3.4) after 24 weeks of therapy (p=0.005) and decreased after 48 weeks to 0.70 SD (range, -4.50 to 2.20) after 48 weeks of therapy.

In two pubertal children a large increase in IGF-1 (by 62 and 42 nmol/l, respectively) and in IGFBP-3 (by 97 and 67 nmol/l, respectively) was observed. Exclusion of these children did not alter the results.

IGF-1, IGFBP-3 and growth

In children with a change in height z-scores above the 75th percentile after 48 weeks, i.e the children with the highest growth velocity from baseline to week 48, a significant larger increase of IGF-1 z-scores (p=0.05) was observed during the first 24 weeks of treatment compared to children with a change in height z-scores below the 75th percentile. Comparison of the changes in IGF-1 z-scores between children with changes of BMI z-scores above and below the 75th percentile also resulted in a significant larger increase of IGF-1 z-scores (p=0.03) during the first 24 weeks of treatment in the children with the highest increase of BMI z-scores. IGFBP-3 z-scores were not different between the two groups.

The median changes in height and BMI z-scores and the differences in changes of IGF-1 and IGFBP-3 between children with a change above and below the 75th percentile are presented in Table 2.

Table 2: Changes in height and BMI z-scores in relation to changes in IGF-1 and IGFBP-3 z-scores

	Change of height z-scores			Change of BMI z-scores		
	> 75th percentile n=12	<75th percentile n=15	p=	> 75th percentile n=6	<75th percentile n=21	p=
Median change of height/BMI z-score	0.46 SD (0.05 to 1.99)	-0.22 SD (-1.2 to 0.03)		1.16 SD (0.91 to 1.25)	-0.1 SD (-3.4 to 0.64)	
Median change of IGF-1 (range)	1.39 SD (0.26 to 3.37)	0.02 SD (-1.27 to 2.09)	0.05	2.7 SD (1.0 to 3.4)	0.5 SD (-1.3 to 2.4)	0.04
Median change of IGFBP-3 (range)	0.7 SD (-0.02 to 3.8)	0.1 SD (-1.4 to 2.4)	0.16	1.6 SD (-0.02 to 3.8)	0.4 SD (-1.4 to 2.5)	0.34

IGF-1, IGFBP3 and immunological status

Although not significantly different, severely immunocompromised children (CDC classification stage 3 (33)) had lower baseline IGF-1 and IGFBP-3 levels compared to children with moderate or no immunodeficiency. In severely immunocompromised children median baseline IGF-1 and IGFBP-3 z-scores were -2.1 and -0.06 SD, respectively, and in children without severe immunodeficiency baseline IGF-1 and IGFBP-3 z-scores were 0.69 and 1.69 SD, respectively. In both groups a significant increase in IGF-1 and IGFBP-3 was observed after 24 weeks of therapy. (Figures 1A and 1B) IGFBP-3 z-scores in severely immunocompromised children were normal (-0.06 SD) whereas IGF-1 z-scores in these children were relatively low (-2.1 SD).

Figure 1A

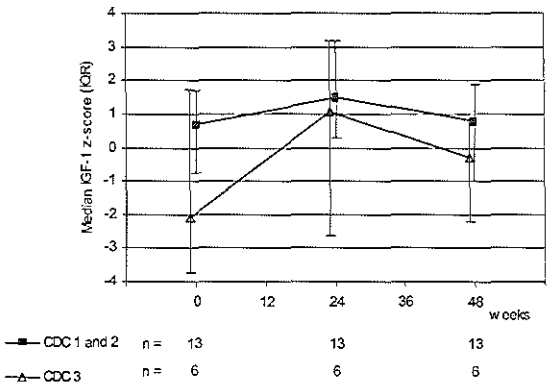

Figure 1B

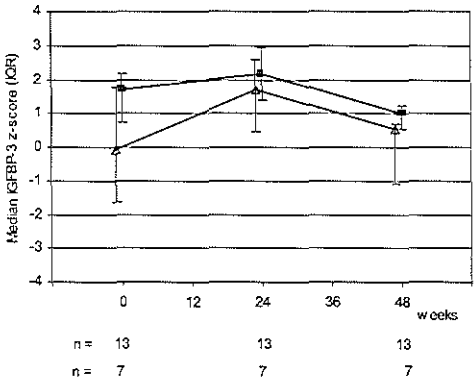

Figure 1 A: *Median IGF-1 z-scores (IQR) and* ***B:*** *median IGFBP-3 z-scores (IQR) from baseline to 48 weeks after the initiation of therapy in patients with no to moderate immunodeficiency (CDC classification stage 1 and 2) and in patients with severe immunodeficiency (CDC classification stage 3).*

IGF-1, IGFBP3 and virological/infectious status

No relation between plasma viral load or TNF-α and IGF-1 or IGFBP-3 levels was found. In addition, no difference in the increase of IGF-1 or IGFBP-3 was found between virological responders and non-responders.

Serum albumin

Since an improvement in the nutritional condition of children can also result in an increase of IGF-1 levels, we analyzed changes in the serum levels of albumin. Serum albumin levels were normal at baseline and further increased after the initiation of therapy from a median (range) of 35 g/l (25-50) at baseline to 42 g/l (26-47) after 48 weeks of therapy.

Discussion

In this study we analyzed changes in endocrinological and immunological factors in relation to catch-up growth after the initiation of HAART in HIV-1 infected children. Most children showed a good clinical, virological and immunological response to the therapy. (20) In contrast to other reports on recovery of growth after the initiation of protease inhibitor containing therapy, in this cohort for the total group no significantly high increases of height and BMI were observed in comparison with age- and sex-matched controls. (23-25) This may be due to the small sample size and to the lack of data on growth before the initiation of therapy. The reports on recovery of growth after the start of treatment with a protease inhibitor containing regimen all compared changes in growth rates between the period before and the period after the initiation of therapy. (23-25)

Endocrine dysregulation seems to play an important role in growth failure in HIV-1 infected children. Several endocrine abnormalities, such as hypothyroidism and abnormal GH and IGF-1 concentrations have been documented. (1, 8) (34-45) The results of our study indicate that baseline

serum levels of IGF-1 and IGFBP-3 are within the range of normal (median: 0.65 SD and 1.4 SD respectively). Serum levels of IGF-1 increased significantly during the first six months of therapy from a median of 13.2 nmol/l at baseline to 22.8 nmol/l after 24 weeks. After 48 weeks IGF-1 levels stabilized to a median of 22.5 nmol/l. Corrected for age and sex, z- scores of IGF-I also increased significantly during therapy from a median of –0.65 SD at baseline to +1.50 SD after 24 weeks of therapy and 0,60 SD after 48 weeks of therapy.

The increases in IGF-1 levels were significantly higher in children in whom the body mass index and length (after correction for age and sex) increased the most.

Serum IGF-1 levels at baseline were relatively low in comparison to baseline levels of IGFBP-3. We hypothesize that this discrepancy is caused by an increased 24-hour GH production. Although we did not measure GH production, the observation that IGFBP-3 levels were normal supports this concept, since IGFBP-3 concentrations are strongly dependent on GH production. The synthesis of IGF-1 by the liver is not only stimulated by GH, but is also dependent on factors within the liver itself, including viral activity, infections, and nutritional status. Since we did not observe a significant improvement in the nutritional status or the extent of the liver function, decreased viral activity and decreased exposure to infections may partly explain the increased levels of IGF-1 during therapy. However, no difference was found between the virological responders and non-responders. Neither did we observe an association between viral load or TNF-α and changes in IGF-1 levels. The combination of relatively high serum levels of IGFBP-3 and low IGF-1 levels suggests the presence of a GH resistant state at baseline. This conclusion is consistent with previous studies. (46, 47) Frost et al. also found elevated GH concentrations and decreased IGF-1 levels. Mulligan et al. demonstrated that the IGF-1 response to exogenously administered GH was blunted in AIDS despite the observation that these patients had normal IGF-1 levels. Similar results have been found in critically ill patients and patients with GH insensitivity syndrome. (48, 49) The normal GH responses to provocative testing in combination with low IGF-1 levels that have previously been reported by Schwartz et al. and Laue et al., could also be explained by GH insensitivity. (1, 43) The decrease in IGFBP-3 and stabilization of IGF-1 levels after an initial significant increase suggests restoration of the sensitivity for GH and a return to an anabolic state.

Arpadi et al. and Pollack et al. reported a strong relation between the rate of growth and the level of HIV-1 replication in HIV-1 infected children. (15, 26) We did not observe an association between virological response and an increase of IGF-1 and IGFBP-3. Although serum levels of TNF-α, a marker of active viral infection, significantly decreased during therapy, TNF-α was also not associated with changes in the levels of IGF-1 or IGFBP-3. This may be explained by the restoration of GH sensitivity during therapy, independent of the level of viral load reduction.

In contrast to other studies that reported thyroid dysfunction in HIV-1 infected children, free T4 levels were normal and did not change during therapy. (1, 8, 35, 37, 43) Morning cortisol levels also were normal. This was also reported by Laue et al. and Schwartz et al.. (1, 43). This suggests that cortisol is not associated with recovery of growth. Although a normal level of cortisol does not imply normal responsiveness of the glucocorticoidreceptor, the absence of elevated levels of cortisol indicates a normal responsiveness of the glucocorticoid-receptor.

In conclusion, we found that baseline serum levels of IGF-1 and IGFBP-3 are within the range of normal, but increased significantly after the initiation of therapy in this cohort with a high percentage of children with an optimal virological response (80%) and a significant decrease of TNF-

α. The combination of relatively high serum levels of IGFBP-3 and low IGF-1 levels suggests the presence of a GH resistant state at baseline. The decrease in IGFBP-3 serum levels and the stabilization of IGF-1 levels after an initial significant increase suggests restoration of the sensitivity to GH and return to an anabolic condition during protease inhibitor containing therapy.
Baseline levels of free T4 and cortisol were within the normal range and did not change during therapy, suggesting that hypothyroidism and adrenal axis abnormalities are not associated with recovery of growth after the initiation of therapy. Further research is needed to investigate whether other factors causing growth failure, such as inadequate caloric intake, abnormalities in lipid metabolism and total energy expenditure, may also play a role in the recovery of growth parameters during HAART and to confirm our hypothesis on the restoration of the sensitivity for GH.

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Part IV

Side effects of highly active antiretroviral therapy

Persistent sterile leucocyturia is associated with impaired renal function in HIV-1 infected children treated with indinavir

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Chapter 14

Abstract

Background: Prolonged administration of indinavir (IDV) is associated with the occurrence of a variety of renal complications in adults. These well documented side-effects have restricted the use of this potent protease inhibitor in children. *Design:* A prospective study to monitor IDV related nephrotoxicity in a cohort of 30 HIV-1 infected children treated with IDV. *Methods:* Urinary pH, albumin, creatinine, the presence of erythrocytes, leucocytes, bacteria, and crystals, and culture were analyzed every three months for 96 weeks. Serum creatinine levels were routinely determined at the same time points. Steady state pharmacokinetics of IDV were done at week 4 after the initiation of IDV. *Results:* The cumulative incidence of persistent sterile leucocyturia (≥75 cells/μL in at least 2 consecutive visits) after 96 weeks was 53%. Persistent sterile leucocyturia was frequently associated with a mild increase in the urine albumin/creatinine ratio and by microscopic hematuria. The cumulative incidence of serum creatinine levels more than 50% above normal was 33% after 96 weeks. Children with persistent sterile leucocyturia more frequently had serum creatinine levels of 50% above normal (p=0.02) than those children without persistent sterile leucocyturia. In children younger than 5.6 years, persistent sterile leucocyturia was significantly more frequent(p= 0.05) than in older children. A higher cumulative incidence of persistent leucocyturia was found in children with an AUC >19 (mg/L*h) or a peak serum level of IDV >12 (mg/l) (p=0.05 and p=0.02, respectively). In 4 children IDV was discontinued because of nephrotoxicity. Subsequently, the serum creatinine levels decreased, the urine albumin/creatinine ratios returned to zero and the leucocyturia disappeared within 3 months. *Conclusion:* Children treated with IDV have a high cumulative incidence of persistent sterile leucocyturia. Children with persistent sterile leucocyturia more frequently had an increase in serum creatinine levels of more than 50% above normal. Younger children have an additional risk for renal complications. The impairment of the renal function in these children occurred in the absence of clinical symptoms of nephrolithiasis. We conclude that IDV associated nephrotoxicity must be monitored closely especially in children with risk factors such as persistent sterile leucocyturia, age <5.6 years, an AUC of IDV >19 (mg/L*h) and a Cmax >12(mg/L).

Introduction

Indinavir (IDV) is a potent HIV-protease inhibitor, which has successfully been used in adults in combination with nucleoside reverse transcriptase inhibitors to suppress infections by HIV-1. Discontinuation of the antiretroviral therapy rapidly results in virological rebound, decreased immune function and the redevelopment of AIDS-defining illness. Thus, antiretroviral drugs such as IDV need to be continued for many years. This necessitates a careful surveillance for long-term toxicity of the medication.

The experience with the administration of IDV to HIV-1 infected children has been limited, because of the absence of a pediatric formulation and the well documented side-effects of the drug on the upper and lower urinary tract in adults. Indinavir is metabolized by the liver, but approximately 20% of a single oral dose is excreted unchanged in the urine. (1) PH dependent crystallization of IDV in renal tubuli may cause renal symptoms such as kidney stones, flank pain (even without evident stone formation), interstitial nephritis, elevation of the serum creatinine, dysuria and asymptomatic urine abnormalities such as hematuria, leucocyturia and crystalluria. IDV

crystals may illicit an inflammatory response in the tubuli, leading to sterile leucocyturia and renal insufficiency. (2-7) To minimalize renal side effects of IDV an increased fluid intake is advised. (8)

The incidence of IDV associated nephrolithiasis in adults varies from 4 to 43%. (2, 9) Renal complications (including nephrolithiasis) by IDV are found in 0 to 80% (10-15) and nephrolithiasis in 0-20% (10, 12-15) of the children who have been treated with this drug.

Renal and urological symptoms of IDV crystals have been correlated with the serum levels of IDV. (16) In children sufficiently high area under the plasma-concentration curves (AUCs) for IDV are required to achieve trough levels (13, 17) which are associated with an optimal virological response. (18-20) The risk of nephrotoxicity in children might therefore be higher than in adults.

In contrast to symptomatic nephrolithiasis, other renal complications such as leucocyturia, microscopic hematuria and crystalluria usually do not lead to a decision to discontinue IDV, although an association between recurrent severe leucocyturia and renal damage by IDV induced crystalluria has been reported in adults. (6, 21)

Currently it is unknown whether and when these asymptomatic signs of renal damage lead to renal complications and long-term renal damage. This prospective study was performed to monitor renal and urinary complications in a cohort of 30 HIV-1 infected children treated with IDV. We hypothesized that IDV related nephrotoxicity might occur more frequently in children than in adults due to the higher risk for cellular damage to the still developing renal system.

Methods

In 1997 a prospective, open, uncontrolled, multicenter study was initiated to evaluate the clinical, immunological and virological response to combination therapy consisting of IDV, zidovudine and lamivudine in HIV-1 infected children (15). Children >3 months of age and one of the following 2 items: a decreased CD4+ T-cell count (<1 year: <1750/mm^3, 1-2 years: <1000/mm^3, 3-6 years: <750/mm^3, >6 years: <500/mm^3) or a HIV-1 RNA load >5000 copies/ml were included. The follow-up period was 96 weeks after the initiation of IDV. Two years after the initiation of this multicenter study a separate study was started in one of the participating centers to analyze additional urinalysis parameters with a follow-up period of 96 weeks.

The Ethics Committee of the University Hospital Rotterdam approved the study. Patients and their caretakers provided written informed consent.

Laboratory parameters

The routinely analyzed laboratory parameters of the children included dipstick analysis for urinary pH (at urine pH values below 5 solubility of indinavir increases (22)), erythrocytes, leukocytes and bacteria at baseline (before the use of IDV) and every three months thereafter. Routine biochemistry tests included serum creatinine. Steady state pharmacokinetics of IDV (400 mg/m^2 q8h) were determined at week 4 after the initiation of IDV. This procedure was repeated when a dosage adjustment of IDV was necessary to normalize the area under the curve-concentration (AUC) curve to adult values (20 mg/L*h, range 10-30 mg/L*h). (17)

Demographic parameters, IDV start date and stop date, IDV dosing regimens, urinary tract symptoms, concomitant treatment, HIV-1 RNA and CD4+ T-cell counts were recorded on structured

data collection forms. Nephrolithiasis related symptoms included renal colic, flank pain, the passing of a stone, and gross hematuria.

Additional laboratory tests were performed in children included in one center, from March 1999 onwards. Urinary pH was measured by means of calibrated electrode technique. Urine albumin and creatinine were measured to calculate the albumin to creatinine ratio as an indicator of renal damage. Urine light microscopy for presence of IDV crystals under a polarized filter was perfomed and urine cultures were performed in patients with a positive dipstick test or microscopy for bacteria.

Sterile leucocyturia is considered to be caused by damage of renal tubuli. Therefore we considered this as the principal endpoint this study. Leukocyturia was defined by the presence of a dipstick test with more than 75 cells/μl. Persistent sterile leukocyturia was present when leukocyturia with negative urine cultures was found at at least two consecutive visits, after the start of IDV. In children with more than 150 leucocytes/μl at at least two visits and a negative urine culture a renal ultrasound was performed.

Analysis

Cumulative incidences of persistent leucocyturia and serum creatinine more than 50% above age and sex specific normal values (23) were calculated with Kaplan-Meier analysis. The influences of pharmacokinetic factors were determined with the logrank test. The relation between the occurrence of persistent leucocyturia and an increased creatinine and between persistent leucocyturia and nephrolithiasis related symptoms were analyzed using the Fisher's exact test. In order to describe urine abnormalities associated with leucocyturia, we performed a cross-sectional analysis 12 weeks after the start of the measurement of the additional urinalysis.

Results

Thirty HIV-1 infected children were enrolled between April 1997 and April 2000. Fifteen children were available for additional analyses between March 1999 and March 2001. The other fifteen children were not available for additional analyses for various reasons: enrollment in another center than the center where the additional analyses were performed (n=8), discontinuation of IDV (n=6: five children because of virological failure and one because of nephrotoxicity) and age > 18 years (n=1). Baseline characteristics of the children are presented in Table 1.

A good clinical, immunological and virological response was observed in all children who were treated with IDV. Most of the children needed 600 mg/m2 q8h of IDV to obtain an AUC of IDV between 10 and 30 mg/L*h. The median (IQR) AUC was 19 (14-28) mg/L*h with a median (IQR) peak level of 9 (6-12) mg/L. Baseline and follow-up serum creatinine and urinalysis data were available from 30 children in whom IDV was initiated.

The cumulative incidence of persistent sterile leucocyturia (≥2 times ≥75 cells/μl)

Eleven out of thirty (37%) children developed persistent sterile leucocyturia (two times or more ≥75 leucocytes/μl). The cumulative incidence after 96 weeks was 53% with a mean time to leucocyturia of 74 weeks (95% confidence interval: 61-87 weeks) of combination therapy containing IDV. (Figure 1A)

The influence of age and sex on the cumulative incidence of persistent sterile leucocyturia was determined. Children were divided into two groups: younger and older than the median age of 5.6 years. Figure 1B shows that children younger than 5.6 years had a significantly higher cumulative incidence of persistent sterile leucocyturia than children older than 5.6 years (p=0.05). Sex did not influence the incidence of leucocyturia.

Table 1: Patient characteristics

Characteristic			
Age (years)	Median (range)	5.6	(2.4-9.9)
Male	N (%)	15	(50)
Body Mass Index (kg/m^2)	Median (IQR)	16	(14-17)
HIV-1 RNA (copies/mL)	Median (IQR)	127,500	(18,400-661,000)
CD4 cells (cells/μL)	Median (IQR)	610	(230-880)
IDV regimen			
• 400 mg/m^2 tid	N (%)	8	(27)
• 500 mg/m^2 tid	N (%)	11	(37)
• 600 mg/m^2 tid	N (%)	7	(23)
• ≥700 mg/m^2 tid	N (%)	4	(13)
• 500/100 mg/m^2 bid*	N (%)		
Serum creatinine (μmol/L)	Median (IQR)	24	(19-38)
Urine			
Leukocytes > 75 cells/μL	N (%)	0	(0)
Erythrocytes > 60 cells/μL	N (%)	0	(0)

*IDV/ritonavir

The cumulative incidence of a change of serum creatinine more than 50% above age and sex specific normal values

Six out of thirty (20%) children had a change of serum creatinine more than 50% above age and sex specific normal values. The cumulative incidence after 96 weeks was 33% with a mean time to creatinine increase of 90 weeks (95% confidence interval: 82-98 weeks) of combination therapy containing IDV. (Figure 1C)

Relation between persistent sterile leucocyturia and an increase of serum creatinine

One of the nineteen (5%) children without persistent sterile leucocyturia had a change in serum creatinine more than 50% above age and sex specific normal values, whereas 5 of 11 (45%) children with persistent sterile leucocyturia had a change in serum creatinine more than 50% above age and sex specific normal values (p=0.02). The median (IQR) time to a creatinine increase among patients with leucocyturia was 24 (0-48) weeks.

Relation between persistent sterile leucocyturia and pharmacokinetic parameters

Children with an AUC_{0-8} of IDV higher than the median AUC of 19 (mg/L*h) had a significantly higher cumulative incidence of persistent sterile leucocyturia compared with those with an AUC ≤19 (mg/L*h) (p=0.05). After 96 weeks 8 of the 12 (67%) children with an AUC>19 (mg/L*h) had persistent sterile leucocyturia, in contrast with 2 of 16 (13%) of the children with an AUC<19 (mg/L*h). The cumulative incidences were 79% and 19%, respectively after 96 weeks. (Figure 1D)

Figure 1A

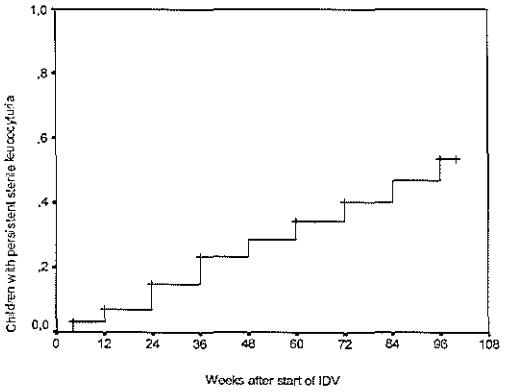

Figure 1B

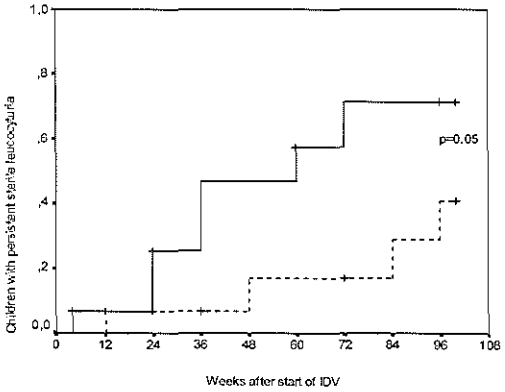

Figure 1C

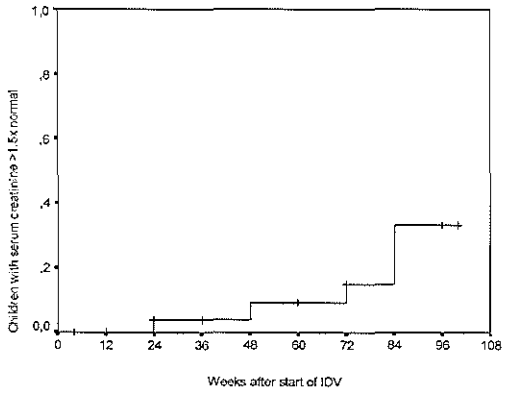

Figure 1D

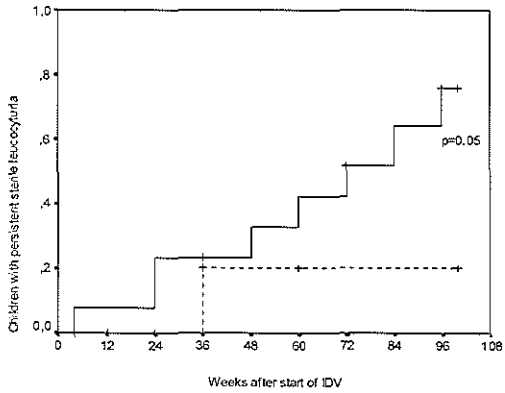

Figure 1E

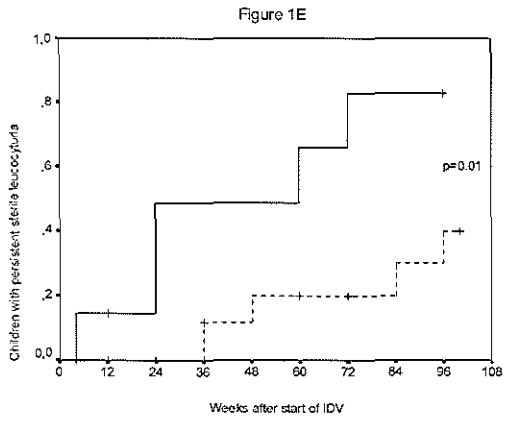

Figure 1A: *Cumulative incidence of persistent sterile leucocyturia.* ***Figure 1B:*** *Cumulative incidence of persistent sterile leucocyturia in children < 5.6 years (dotted line) and in children >5.6years.* ***Figure 1C:*** *Cumulative incidence of children with creatinine increase ≥ 50% above age and sex specific normal values.* ***Figure 1D:*** *Cumulative incidence of persistent sterile leucocyturia in children with AUC of IDV ≤19 (dotted line) or >19 (mg/L*h).* ***Figure 1E:*** *Cumulative incidence of persistent sterile leucocyturia in children with Cmax ≤12 (dotted line) or >12 (mg/L).*

Relation between persistent sterile leucocyturia and nephrolithiasis related symptoms

Four of the 21 (19%) children without persistent sterile leucocyturia presented with urological symptoms, whereas 7 of 9 (78%) children with persistent sterile leucocyturia had symptoms during the follow-up time (p=0.003).

Hematuria

Persistent hematuria (≥2x ≥60 cells/μl) was not detected in any child.

In addition to the standard analyses performed in the 30 children, urine creatinine, urine albumin, quantitative pH measurements and crystalluria were analysed in 15 children. At the time of the start of the additional analyses, these children were using IDV for a median of 75 weeks (interquartile range (IQR): 8-77 weeks).

A cross-sectional analysis at week 12 after the start of the initiation of additional analysis showed that in these children (median time on IDV (IQR): 87 (20-89) weeks) 33% of the patients had a change of serum creatinine more than 50% above age and sex specific normal values, 43% of the patients had leucocyturia, 21% had microscopic hematuria, 54% had crystalluria and 29% had an albumin/creatinine ratio ≥ 3.5 g/mmol. Urine cultures were all negative for bacteria.

In Figure 2 urine abnormalities associated with leucocyturia at week 12 are presented. An increase of serum creatinine levels of more than 50% above age and sex specific normal values, an albumin/creatinine ratio ≥ 3.5 g/mmol and hematuria were observed more frequently in children with leucocyturia. The median (IQR) albumin/creatinine ratio of children with and without persistent leucocyturia was 0.7 (0.6-2.5) and 2.8 (1.2-10.6) respectively. In contrast IDV crystalluria was not detected more frequently in children with leucocyturia. The presence of symptoms, urinary pH>5, and the presence of crystalluria were not associated with persistent sterile leucocyturia.

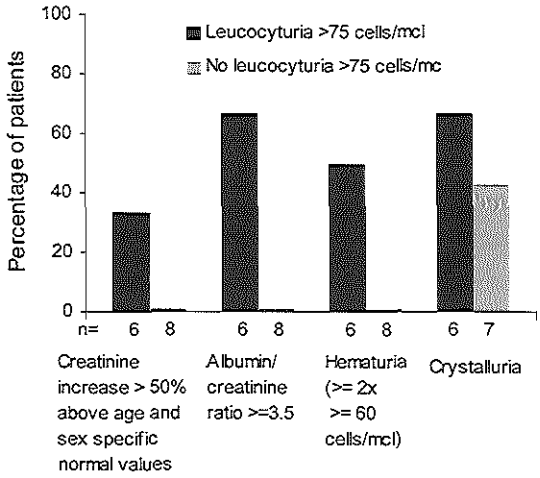

Figure 2: *Urinary abnormalities associated with leucocyturia (cross-sectional week 12) in 15 children included in cohort B*

Symptoms of nephrotoxicity after discontinuation of IDV because of nephrotoxicity

In four of the fifteen children IDV was discontinued because of nephrotoxic symptoms (n=2) or nephrolithiasis on renal ultrasound (n=2). In these children serum creatinine (µmol/l) levels decreased from a median (IQR) of 54 (49-75) at the last observation during the use of IDV to 39 (28-42) 12 weeks after discontinuation of IDV (p=0.07). The albumin/creatinine ratio decreased from 16 (8-44) to 0.7 (0.4-2.1) g/mmol (p=0.07). Leucocyturia disappeared within 3 months after the discontinuation of IDV. Figure 3 shows the serum creatinine levels and the albumin/creatinin ratios of the four children that discontinued IDV because of nephrotoxicity. Urine albumin/creatinine ratio increases preceded serum creatinine increases and may therefore may be an early marker of renal impairment.

In three other children that discontinued IDV for other reasons (virological failure, n=2 and because of the poor taste of IDV, n=1) serum creatinine levels did not decrease, whereas the albumin/creatinine ratio showed a decrease from 3.94 (0.51-11.2) to 0 g/mmol (p=0.10).

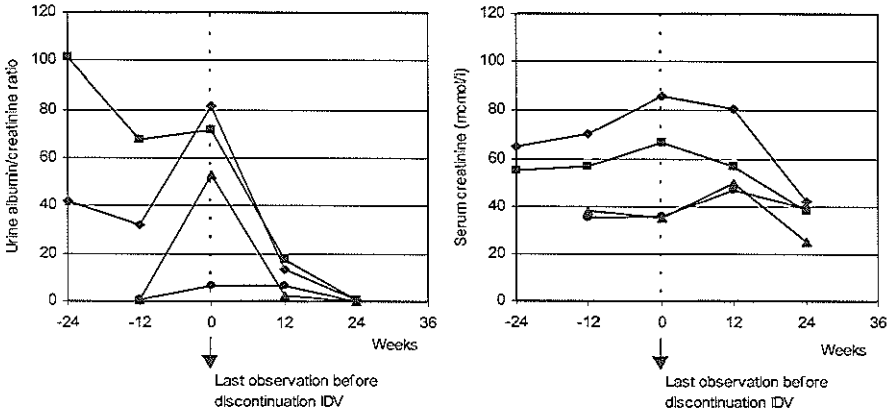

Figure 3: *Urine albumin/creatinine ratio (g/mmol) and creatinine levels (mcmol/l) during the use of IDV and afer discontinuation of IDV in 4 children.*

Discussion

We here present the first study to monitor nephrotoxicity in HIV-1 infected children with a prolonged treatment with IDV. We hypothesized that IDV related nephrotoxicity might occur more frequently in children than in adults due to the higher risk for cellular damage to the still developing renal system.

In our study a cumulative incidence of persistent sterile leucocyturia (≥2x ≥ 75 cells/µl) of 53% was observed after 96 weeks. This persistent sterile leucocyturia was frequently accompanied by a mild increase of the urine albumin/creatinine ratio and microscopic hematuria. The cumulative incidence of an increase in serum creatinine levels more than 50% above normal was 35% after 96 weeks. Children with persistent sterile leucocyturia more frequently had an increase of serum creatinine levels of more than 50% above normal (p=0.02). This suggests that persistent sterile leucocyturia is an early indication for the development of renal damage.

Recently Gagnon et al. found a significant reduction in the renal function of 3 adults with recurrent severe leucocyturia. Our data confirm the observations in adults of Gagnon et al., that reduction of renal function is associated with recurrent severe leucocyturia, but not with isolated hematuria or crystalluria. (21) Renal damage resulting in leucocyturia and an increased creatinine may be a result of irritation of the tubular epithelium. This is supported by the pathologic finding of tubulointerstitial nephritis in patients on therapy with IDV. (4, 24, 25)

The prevalence of persistent leucocyturia in adults screened on the same nephrotoxicity monitoring program was 22% (personal communication), which is substantially lower than we observed in children. One might hypothesize that IDV crystals more easily congest in the small tubuli of young children which may lead to a higher incidence of nephrolithiasis. The more frequent presence of persistent sterile leucocyturia in younger children confirms this observation. However, nephrolithiasis was only diagnosed by renal ultrasound in two asymptomatic children with persistent sterile leucocyturia (26). Renal ultrasounds of the other children with persistent leucocyturia showed no nephrolithiasis. Since it is well documented that the occurrence of IDV nephrolithiasis increases with a poor hydration status and high environmental temperatures (27), the Dutch climate with relatively moderate temperatures may contribute to a lower incidence of nephrolithiasis in our patients. Persistent leucocyturia might have been prevented by an increased fluid intake. Since it is more difficult to achieve a large fluid intake in young children, a relatively small fluid intake in younger children may be the cause of the more frequent occurrence of persistent sterile leucocyturia in children younger than 5.6 years (cumulative incidence after 96 weeks: 78%).

We did not observe an association between IDV crystalluria and leucocyturia. Since IDV crystals can develop in the urine canister (28), it is possible that crystalluria reflects the time lapse between urine collection and urinalysis.

We observed a higher cumulative incidence of persistent leucocyturia in children with an $AUC_{0\text{-}8}$ of IDV of >19 (mg/L*h) and in children with a peak level of IDV higher than 12 (mg/L). This is in accordance with previous publications on the relation between levels of IDV and urological complications in adults. An AUC of IDV less than 20 (mg/L*h) is associated with virological failure (17). This observation complicates the treatment of HIV-1 infected children with IDV: to achieve optimal virological suppression an AUC higher than 20 (mg/L*h) is required, but to avoid persistent leucocyturia an AUC less than 19 (mg/L*h) is needed.

These observations suggest that IDV may be less useful in the treatment of HIV-1 infected children. However, IDV is a very potent protease inhibitor which in combination with nucleoside analogues gives an excellent long-term clinical, virological and immunological response in adults and in children. (15, 29, 30) We therefore propose to monitor nephrotoxicity very closely in children treated with IDV and change therapy only in the case of overt signs of renal impairment. In this respect it is reassuring that the signs of renal impairment are reversible after discontinuation of IDV. Serum creatinine levels decreased in the four children with signs of nephrotoxicity which discontinued IDV. The urine albumin/creatinine ratio returned to zero in all patients. It still remains unclear whether renal impairment is reversible in all stages of damage or that a chronic renal insufficiency will develop above a critical level of cellular damage.

We conclude that prolonged therapy with IDV is associated with a high risk for persistent sterile leucocyturia in children especially in those younger than 5.6 years. The presence of sterile leucocyturia is associated with a significant increase in serum creatinine levels. A high AUC (>19

(mg/L*h)) and high peak levels (>12 (mg/L) of IDV are associated with the occurrence of leucocyturia. Therapeutic drug monitoring of IDV serum levels is therefore essential to estimate the risk of nephrotoxicity. Children with risk factors for the development of nephrotoxicity such as an age <5.6 years, AUC of IDV> 19 (mg/L*h), Cmax >12 (mg/L)) should be monitored routinely by means of urinalysis and analysis of serum creatinine levels.

Acknowledgements

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Indinavir associated asymptomatic nephrolithiasis and renal cortex atrophy in two HIV-1 infected children

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AIDS 2001;15:1745-7

Chapter 15

Case reports

A 3-year old girl diagnosed with HIV-1/AIDS was treated with indinavir (500 mg/m^2 q8h), zidovudine (120 mg/m^2 q8h) and lamivudine (4 mg/kg q12h) because of a high viral load (33,100 copies/ml). Pharmacokinetic analysis of indinavir showed a large area under the curve-concentration (AUC) and a high Cmax: 39 h*mg/L and 13.55 mg/l respectively. The dosage of indinavir was not reduced because of the absence of clinically overt toxicity. The girl subsequently showed a good virological and immunological response to therapy. Urinalysis before the start of therapy was normal. Leukocyturia (51-250 leukocytes/μl), without proteinuria, hematuria or crystalluria was detected 9 months after initiation of therapy and persisted during the next 1½ years. Urine cultures were repeatedly negative. There were no clinical symptoms of nephrotoxicity (flank pain, renal colic). Renal and liver function tests were normal. A renal ultrasound was performed after 27 months of therapy because of the persistent leucocyturia and showed medullary calcifications and cortical atrophy in both kidneys. Indinavir was subsequently switched to nelfinavir. The fluid intake was increased to increase the solubility of indinavir in the urine. In addition, vitamin C (15 mg/kg q6h) was administered because of a potential favourable influence on the solubility of indinavir in urine (at urine pH values below 5 solubility of indinavir increases (1)). Three months later, leukocyturia decreased to 0-10 leukocytes/μl and a renal ultrasound showed a partial resolution of medullary calcifications and a normal cortex.

A 4-year old girl with HIV-1/AIDS started HIV-treatment with indinavir (500 mg/m^2 q8h), zidovudine (120 mg/m^2 q8h) and lamivudine (4 mg/kg q12h) in 1997. The AUC and Cmax of indinavir were higher as compared to normal values for adults: 27 h*mg/L and 16.27 mg/l respectively. The dosage of indinavir was not decreased because of the absence of clinical signs of toxicity. Urinalysis before the initiation of therapy was normal. After 3 years of therapy a renal ultrasound was performed because of a persistent sterile leukocyturia (>250 leukocytes/μl) and microscopic hematuria (11-50 erythrocytes/μl) without other urinary complications or laboratory abnormalities. This revealed multiple calcifications in the papillae and cortical atrophy of both kidneys. (Figure 1) Indinavir was switched to nelfinavir and the fluid intake was increased. Vitamin C (15 mg/kg q6h) was administered.

Three months later, leukocyturia and microscopic hematuria decreased to 0-10 leukocytes/μl and 0-10 erythrocytes/μl respectively and the renal ultrasound had improved. A decrease of medullary calcifications and a normal cortex was observed.

Indinavir is a potent HIV-protease inhibitor, which is widely used in combination with nucleoside reverse transcriptase inhibitors to treat HIV-1 infected adults and children. Indinavir crystals cause nephrolithiasis in 4-28% of patients, who typically present with flank pain and microscopic hematuria. (2,3) It is not yet clear, whether indinavir induced urine abnormalities in asymptomatic patients with a normal renal function should have consequences for continuation or dosage adjustment of this therapy. Gagnon et al. suggested that recurrent severe leukocyturia in adults may be an indicator of renal damage by indinavir, even in the absence of crystalluria. However, three of their five patients with leukocyturia had a significantly increased serum creatinine. (4) The renal ultrasound of our patients showed severe cortical atrophy and extensive calcifications despite the absence of an increase in serum creatinine. We observed that these abnormalities were reversible

after discontinuation of indinavir, increased fluid intake and administering vitamin C. Nevertheless we cannot exclude that this would also be the case in a more advanced stage of renal damage.

Elevated plasma concentrations of indinavir have been associated with urological complications (renal colic, flank pain or haematuria) (5). The high AUC and/or the high Cmax may thus have contributed to the nephrotoxicity in these two children. We therefore advise to routinely monitor plasma concentrations of indinavir in children and to adjust the dosage in order to reach normal adult plasma concentrations. In addition we propose to regularly perform renal ultrasounds in children who are treated with indinavir and who have an abnormal urinalysis.

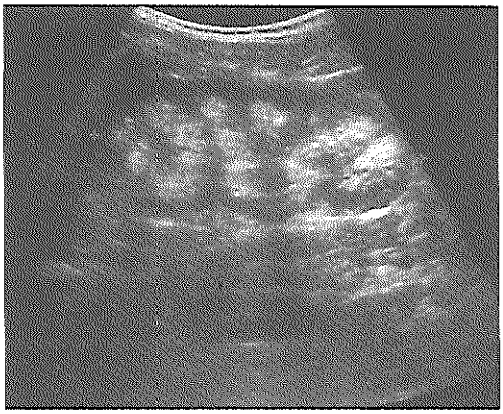

Figure 1 *In a 4-year-old girl undergoing treatment with indinavir, renal ultrasound was performed because of persistent leukocyturia. There were no clinical symptoms of nephrolithiasis and renal function was normal. Ultrasound of the right kidney shows calcifications in all papillae and a decreased thickness of the cortex, maximum of 0.3 cm.*

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Increased cholesterol and normal glucose levels in HIV-1-infected children treated with protease inhibitors

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Arch. Dis. Child.: in press

Chapter 16

Abstract

Background: The use of protease inhibitors (PIs) in patients with HIV-1/ AIDS has been associated with peripheral lipodystrophy, hyperlipidemia and insulin resistance. To date, all studies in this field have been performed in adults, whereas no data are available in children. *Objective:* To evaluate the influence of 18 months or more of highly active antiretroviral therapy (HAART), using one protease inhibitor and two nucleoside-analogue reverse transcriptase inhibitors, on the serum levels of lipids and glucose in HIV-1-infected children. *Methods:* Twenty children were included. Serum levels of fasting triglyceride, total cholesterol, high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL) and free fatty acids were evaluated at two timepoints: within the first month of HAART and at follow-up, after 18 months or more. Serum levels of fasting glucose, insulin and C-peptide were only evaluated at follow-up. *Results:* After 18 months or more of HAART serum levels of total cholesterol, HDL and LDL were significantly increased compared with baseline values. The total cholesterol/ HDL ratio, triglyceride and free fatty acid concentrations remained stable over time. The fasting glucose, insulin and C-peptide serum levels were normal. *Conclusion:* Lipid abnormalities similar to those seen in adults treated with protease inhibitors are also observed in HIV-1-infected children.

Introduction

Protease inhibitors (PIs) have made a major contribution to the successful management of patients with HIV-1/ AIDS. Administration of PI containing regimens results in an improvement of virological and immunological parameters, clinical outcome and survival (1-3).

Since the registration of PIs in 1996, a syndrome of hyperlipidemia, insulin resistance and peripheral lipodystrophy has been documented in HIV-1-infected adults (4, 5). The pathogenesis of this syndrome remains unclear. It is still unknown whether these abnormalities are caused by PIs or that the effect of other antiretroviral drugs may also play a role. Recently, Brinkman and colleagues hypothesized that mitochondrial toxicity induced by nucleoside-analogue reverse transcriptase inhibitors may be a key factor in the pathogenesis of lipodystrophy (6).

The prevalence of this syndrome is still unknown. Many investigations reported only the prevalence of one aspect of the syndrome, since the three major symptoms (hyperlipidemia, insulin resistance and peripheral lipodystrophy) do not coexist in all patients and can be encountered in various combinations. The prevalence of insulin resistance and of lipodystrophy in PI recipients, after a median or a mean period of fourteen months of PI use, has been estimated at 61% and 64% respectively (4, 5). An important factor in the analysis of the prevalence of lipodystrophy is the timing of the syndrome in relation to the start of antiretroviral therapy (7). Studies with a longer follow-up report a higher prevalence than those with a short follow-up period.

Currently only two reports are available on the prevalence of this syndrome among children (8, 9). In one study abnormal body-fat distribution was seen in 1.5 % of 1644 children receiving a PI, versus 0.4% of 1069 children with therapy without PIs (8). In our study, in twenty children with prolonged therapy (> 18 months) with HAART, we did not observe clinical symptoms of lipodystrophy. However, we did not use standardised measurements, like waist/ hip ratio, skinfold thickness measurements and dual-energy x-ray absorptiometry. Watson and colleagues measured

the serum cholesterol levels in children and found a rise in mean non-fasting levels from 3.38 mmol/l before to 4.68 mmol/l after (a not specified period of) PI use (9).

The aim of the current study was to evaluate the influence of HAART on serum levels of lipids and glucose in HIV-1-infected children treated for more than 18 months with an indinavir (IDV) or nelfinavir (NFV) containing regimen of HAART.

Methods

We analysed relevant laboratory data from the Dutch prospective open uncontrolled multicentre study on the efficacy of HAART in HIV-1-infected children (10).

Serum levels of fasting triglyceride, total cholesterol, HDL, LDL and free fatty acids were evaluated at two timepoints: within the first month of HAART (with the exception of two children) and at follow-up, after 18 months or more. In this article the values within the first month of HAART will be indicated as 'baseline values'. Serum levels of fasting glucose, insulin and C-peptide were only evaluated at follow-up. These laboratory parameters were not stable enough to be analysed retrospectively.

Patients

Children between the ages of 3 months and 18 years were eligible for enrolment in the Dutch multicentre study. Children were included when plasma HIV-1 RNA tests were positive on two subsequent occasions (children less than 18 months old) or when the HIV serology was positive (children more than 18 months old) in the presence of one of the following abnormal test results: a mean HIV-1 viral load of more than 5000 copies/ml (mean of two measurements with less than four weeks in between) and/or a CD4 cell count below 1 year: $<1750/mm^3$, 1-2 years: $<1000/mm^3$, 3-6 years: $<750/mm^3$, >6 years: $<500/mm^3$. Patients were excluded when they had been pretreated with antiviral agents other than zidovudine and/or didanosine or zalcitabine. There were no restrictions with regard to gender, ethnicity, or route of acquisition of HIV-1 infection.

Additional inclusion criteria for this study were: the use of HAART for 18 months or more, no use of corticosteroids, fasting values at baseline and a fasting condition at follow-up. Fasting was defined as a fasting period of at least three hours.

Laboratory analysis

Serum was obtained within the first month of HAART after a 12 h overnight fasting period and was stored at -80°C. Serum triglyceride, total cholesterol, HDL and free fatty acid levels were determined retrospectively by a commercial photoenzymatic assay (Hitachi, Boehringer-Mannheim, Germany).
The second timepoint of blood sampling was after 18 months or more of HAART. These samples were taken after a fasting period of at least three hours. Triglyceride and total cholesterol were determined using another commercial photoenzymatic assay (Dimension, Dade, USA); free fatty acids and HDL using Hitachi (Boehringer-Mannheim, Germany). Serum levels of LDL cholesterol were calculated by the method of Friedewald *et al* (11).

Glucose levels were determined by a commercial glucose hexokinase assay (Dimension, Dade, USA). Serum insulin and C-peptide levels were determined by a commercial radioimmunoassay (Medgenix, Biosource, Belgium).

Statistical analysis

Statistical calculations were performed with the SPSS statistical analysis software program (version 8.0). Comparison of lipid levels at both timepoints was performed by the Wilcoxon signed rank test. Correlation of lipid, glucose, insulin and C-peptide levels at follow-up was assessed using the Spearman rank correlation test. Comparison of lipid levels at baseline in pretreated and naive children was performed using the Mann-Whitney U-test. P values of $< 0,05$ were considered to indicate statistical significance.

Results

Twenty HIV-1-infected children, nine boys and eleven girls, were included in this study. Twelve children received IDV from the beginning, whereas two children were treated with NFV. Five children switched from IDV to NFV treatment during the course of these 18 months, because of lack of tolerance for IDV. One child changed to a combination of IDV plus ritonavir (RTV) after twelve months of IDV use, because of virological failure. Additional patient characteristics at both timepoints are indicated in Table 1.

Serum lipid levels of total cholesterol, HDL and LDL were significantly increased after 18 months or more of HAART (p values: <0.001). The total cholesterol/HDL ratio, fasting triglyceride and free fatty acids levels remained stable over time (Table 2).

A high fasting triglyceride concentration of 2.8 mmol/l was found in one patient, who used a combination of IDV and RTV. In this child the fasting triglyceride concentrations within the first month of IDV use and before the switch to a combination of IDV and RTV were 0.7 mmol/l and 1.3 mmol/l respectively.

Comparison of the serum lipid levels within the first month of HAART in pretreated and naive children showed significantly lower serum levels of total cholesterol and LDL in pretreated children (p values: 0.031 and 0.025 respectively).

Two patients had fasting glucose concentrations of more than 7.1 mmol/l at follow-up. Serum glucose levels were normal after an oral glucose tolerance test in the first patient. In the other patient an oral glucose tolerance test was not done.

The Spearman Correlation Test showed a negative correlation between the serum levels of free fatty acids and glucose, insulin and C-peptide at follow-up (coefficient: -0.52, -0.77 and -0.87; p-values: 0.032, 0.001 and 0.001, respectively).

Discussion

Since the registration of PIs in 1996 a syndrome of hyperlipidemia, insulin resistance and peripheral lipodystrophy has been reported in HIV-1-infected adults. Several reports have been published about the cause, pathogenesis, severity and prevalence of this syndrome(6, 12, 13). However thus far, these studies were only performed in adults. This is the first report in HIV-1-infected children, in which increased cholesterol levels and normal glucose tolerance are observed.

The aim of this study was to evaluate serum lipids and glucose after 18 months or more of HAART in children with HIV-1-infection. This is more complex than the evaluation of these laboratory parameters in adults, because levels of glucose, insulin and lipids are age-dependent (14-16).

Table 1: Patient characteristics within first month of HAART and at follow-up

	First timepoint (≤1 month of HAART) (n=20)	*Second timepoint (≥18 months of HAART) (n=20)*
Demography and antropometry		
Sex: Male/ female	*9/ 11*	
Race: - African	*12*	
- Caucasian	*4*	
- Asian	*1*	
- Other	*3*	
Age (years)	*5.8 (1.1-16.5)*	*7.3 (2.8-18.2)*
Standard deviation (z-scores) of BMI	*0.12 (-2.9-1.4)*	*-0.02 (-2.1-2.0)*
Clinical stage (CDC-classification) at start HAART:*		
N1/N2	*2/1*	
A1/A2/A3	*2/4/3*	
B1/B2	*1/3*	
C2/C3	*1/3*	
Prior treatment		
No prior treatment	*9*	
Prior treatment: number (duration of use in months):		
- Zidovudine	*9 (21, range: 8-118)*	
- Zidovudine + Zalcitabine	*1 (18)*	
- Zidovudine + Didanosine	*1 (21)*	
Surrogate markers		
CD-4 cell percentage (%)	*27 (1-72)*	*37 (18-55)*
HIV-1 RNA (copies/ml)	*715 (480-11800)*	*500 (500-67600)*
Protease inhibitor		
Indinavir	*18*	*12*
Nelfinavir	*2*	*7*
Indinavir + Ritonavir	*0*	*1*
Duration of protease inhibitor use (months)	*1 (0.25-3)*	*22 (18-24)*
Combination of reverse transcriptase inhibitors		
Zidovudine + Lamivudine	*18*	*14*
Didanosine + Stavudine	*1*	*4*
Lamivudine + Stavudine	*1*	*2*

Data are numbers or medians (ranges) *Clinical and Immunologic catagories as defined by the US Centers for Disease Control and prevention (CDC) (22)

Despite the additional complexity in the analysis, we found a significant increase in serum levels of total cholesterol, HDL and LDL after 18 months or more of HAART in HIV-1-infected children.

To date it has not been clarified whether these metabolic changes are the result of HAART or whether the HIV-1-infection itself is responsible. Hypertriglyceridemia and low levels of total cholesterol, HDL and LDL have been detected in HIV-1-infected patients without prior antiretroviral

therapy, especially in the late phase of the disease (17). Therefore, one would expect a more

Table 2: Metabolic data of 20 children using HAART

Metabolic parameters	*First timepoint (≤1 month of HAART)*	*Second timepoint (≥18 months of HAART)*	*P-value*
Total cholesterol (mmol/l)	*3.0 (1.7-4.3)*	*4.7 (3.2-6.5)*	*0.001*
HDL (mmol/l)	*0.8 (0.4-1.2)*	*1.3 (0.7-2.5)*	*0.001*
LDL (mmol/l)	*1.7 (0.8-4.6)*	*2.7 (1.7-4.9)*	*0.001*
Cholesterol/ HDL ratio	*3.6 (2.2-6.5)*	*3.4 (2.2-6.6)*	*0.514*
Triglyceride (mmol/l)	*0.5 (0.2-2.2)*	*0.8 (0.1-2.8)*	*0.167*
Free fatty acids (mmol/l)	*0.6 (0.2-1.3)*	*0.5 (0.1-2.0)*	*0.808*
Glucose (mmol/l)	*NA*	*5.0 (3.7-7.4)*	
Insulin (mU/l)	*NA*	*11 (5-72) **	
C-peptide (mmol/l)	*NA*	*0.7 (0.3-3.4) **	

Data are medians (ranges) NA: not available * Data not available for one patient

significant rise in levels of total cholesterol, HDL and LDL in patients with an advanced stage of disease, who are succesfully treated with HAART, as compared to children in an early phase of HIV/ AIDS. Thus, the significant rise in total cholesterol, HDL and LDL in children with HIV/ AIDS may not only be attributed to the effects of HAART, but may be also partially be the result of a normalisation of pre-existing lipid abnormalities.

An explanation for the absence of a significant rise in serum triglycerides (and at the same time a limitation of the design of this study) is that we did not collect fasting values of serum lipids before the start of HAART. Churchill *et al* have documented, that serum triglyceride levels have a normal course with a peak after four weeks of PI use (18). Thus, it may be possible that we have missed the peak at our first timepoint.

It is difficult to discriminate the metabolic effects of PIs from those of other antiretroviral drugs in this study. Most children received a combination of a PI, zidovudine and lamivudine. Zidovudine and lamivudine are also reported to cause lipodystrophy and lipid abnormalities (19). Before starting HAART, eleven children were pretreated with zidovudine. These pretreated children had significantly lower levels of total cholesterol and LDL at baseline than naive children, suggesting that zidovudine itself may have an effect on the lipid metabolism.

Although our patient group was too small to evaluate any differences between the various PIs on the selected metabolic parameters, we observed a high triglyceride level of 2.78 mmol/l in one patient receiving a combination of IDV and RTV. Similar to other studies, this may suggest that RTV is a more likely cause of elevated triglyceride levels than other PIs. (4, 20)

Our data show that the fasting glucose, insulin and C-peptide values were normal. Unfortunately these values were only analysed at follow-up. Therefore we could not confirm a potential rise in serum levels. In two children a fasting glucose concentration of more than 7.1 mmol/l was detected. However, one child did have a normal oral glucose tolerance test, whereas we did not perform this test in the other child. These high values of glucose may have been coincidental. Alternatively, these patients may have taken food in the three hours fasting period.

Further investigations are necessary to determine, whether insulin resistance is present in HIV-1-infected children on HAART.

In summary, we found an increase in serum levels of total cholesterol and LDL after PI use in HIV-1-infected children, as was previously observed in adults (4, 21). However in contrast with adults, a marked increase in HDL, but normal glucose were observed (5, 13).

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Summary and discussion

Chapter 17

In **Chapter 1** we describe the background and initial goals of the studies presented in this thesis. In 1997, the increasing number of children with HIV/AIDS in The Netherlands stimulated three university children's hospitals (in Amsterdam, Rotterdam and Utrecht) to coordinate their efforts to optimize the diagnosis, therapy and care of these children. As part of this initiative a multidisciplinary working group was formed. At the same time a collaborative open-label multicenter trial was started. The aim of this study was to evaluate the clinical effects, the virological response rate, pharmacokinetic and pharmacodynamic aspects of selected antiretroviral agents and the extent of immunereconstitution in response to combination therapy consisting of indinavir, zidovudine and lamivudine. A team of experts in immunology, virology, pharmacology, internal medicine and pediatrics specialized in HIV/AIDS was created in order to rapidly apply new concepts in the field of care, cure and research in adults into the field of pediatrics. In addition, a multidisciplinary team was created to provide care for children with HIV/AIDS in the Sophia Children's Hospital in Rotterdam. This team includes nurse specialists, a social worker, research physicians and pediatric infectious disease experts. The leading principle in the design of the study was that care of HIV infected children should be organized in a structured scientific way and that research projects should predominantly serve to improve the quality of life of children with HIV/AIDS.

In **chapter 2** we present an overview of the content of this thesis, which contains the results of a comprehensive set of studies on the epidemiology of HIV-1 infection in children in The Netherlands, the effectiveness of prevention of transmission of infection from mother to child, the pharmacokinetic and pharmacodynamic aspects of HAART in children, the effects of HAART on the well-being and growth of children, selected aspects of the immunereconstitution, which is associated with the use of HAART in children and prospective analyses of side-effects of HAART.

In 1995 a prospective surveillance of all children who were pre- or perinatally exposed to HIV or postnatally diagnosed with HIV infection was started in The Netherlands. Pediatricians reported new children monthly to the Dutch Pediatric Surveillance Unit. In **chapter 3** we show the effects of implementation of the guidelines to prevent perinatal transmission in The Netherlands in the period from 1995 until 2000. An increase in the number of children who were perinatally exposed to HIV was observed and the percentage of children who became infected after exposure simultaneously decreased from 20% in 1995 to only 4% in 1999. This improvement reflects the increased understanding of the pathogenesis and treatment of HIV infection and of the mode of prevention of mother-to-child transmission. The risk on mother-to-child transmission, which is 15-30% without interventions (1-4), can be reduced to 0-3% by the administration of highly active antiretroviral medication to mother and by the administration of zidovudine to the child during the first weeks of life. When the maternal viral load is above 1000 copies/ml at the time of delivery an elective cesarean section should be performed, which may in combination with antiretroviral prophylaxis reduce the risk for transmission to almost 0%. (5-9) The decrease in transmission from 20% to 4% can be attributed to a great extent to the increase in the use of HAART by pregnant HIV-1 infected women in this period from 0% to 70%. We therefore conclude that the Dutch guidelines to prevent mother-to-child transmission of HIV-1 infection have been well implemented and are efficacious.
In **chapter 4** we present data on the cumulative number of children living in The Netherlands who were diagnosed with HIV infection, on their mode of transmission, their risk factors and their clinical features from the start of the AIDS epidemic in the 1980's until 2000. The increase in the number of

newly detected HIV-1 infected children during the period 1995-1997 reached a plateau during the period 1998-2000. This may be due to a more active approach in the counseling and screening for HIV in pregnant women. Since 1999 women at risk for infection with HIV (women originating form a country with a generalized HIV epidemic and women having sexual contacts with men at risk for infection with HIV) are counseled and advised to undergo an HIV test. In 86% of the children one or both parents originated from a country with a generalized HIV epidemic. Most children (81%) were infected by mother-to-child transmission. Fourty-eight percent was born in The Netherlands. The risk for transmission of HIV could be significantly reduced when the mother was tested prenatally for HIV and treated according to the guidelines. In 2000 Postma et al. evaluated the cost-effectiveness of universal HIV-screening of pregnant women in Amsterdam. (10) They showed a favorable cost-effectiveness of universal HIV-screening of pregnant women. However, the actual costs of the treatment of HIV infected children are not known. Therfore, accurate estimates of the cost-benefit analysis are not yet possible. We have therefore recently initiated a study to analyze the costs for the care and treatment of HIV-1 infected children in The Netherlands in collaboration with Postma. Our data indicate that one or both parents of 86% of the children originate from a country with a generalized HIV epidemic. It therefore seems unnecessary to screen all pregnant women in The Netherlands as was recently advised by the Ministry of Health. A screening program in individuals belonging to risk-groups is in our opinion a much more cost-effective solution.

One or both parents of 95% of the newly detected HIV-1 infected children originated from other countries than The Netherlands. The treatment of these children is complicated by difficulties in communication and by major differences between the families of these children and the indigenous Dutch population in social, cultural and economical aspects. These differences demand a very intensive and family specific approach with attention for the transcultural aspects. It is obvious that this very intensive approach is associated with substantially higher costs in the care for HIV infected children, than in the care for adults with HIV/AIDS.

It has been well known that the pharmacokinetics and pharmacodynamic aspects of many drugs are very different in children as compared to those in adults. One can therefore not assume that successful medical treatment in adults will be always beneficial in children. Therefore, the administration to children of new agents such as the HIV-1 protease inhibitors, should be preceded by detailed pharmacokinetic and pharmacodynamic evaluations. The goal of this approach is to ensure similar efficacy and equal or less toxicity compared to those reported in adults. In **chapter 5**, the pharmacokinetics of the protease inhibitor indinavir in children are described. We showed the presence of substantial interindividual differences in the different pharmacokinetic parameters such as AUC, Cmax and Cmin. The higher metabolic clearance and the increased volume of distribution of indinavir resulted in suboptimal drug levels in many of the HIV-1 infected children. A number of studies previously showed a relation between the concentration of indinavir and the virological response rate. (11-13)

We describe in chapter 5 the presence of a relation between an AUC of indinavir above 20 mg/l*h and the virological success rate. Because of the major age-related individual differences in the pharmacokinetics of indinavir, one needs to monitor the levels of indinavir in plasma shortly after the start of an indinavir containing regimen and to adjust the indinavir dose when necessary in stead of using a fixed dosis regimen for each child. There has been some concern that the risk of indinavir-

induced nephrological toxicity (kidney stones, hematuria, and flank pain) in children may be higher than in adults. Urological toxicity is the result of precipitation of indinavir crystals and this is correlated with the peak level of indinavir in plasma. (14) Although indinavir peak levels were higher in children compared to adults none of the children developed kidney stones within the first 6 months of treatment. However, in chapters 14 and 15 nephrological side-effects of indinavir in children were analyzed in more detail and were found to be present in a high percentage of the children. In addition, a relation between drug levels and the incidence of nephrological toxicity was observed.

The unpredictable interindividual differences in the pharmacokinetics of protease inhibitors (and probably also other antiretroviral drugs) in children underscore the importance of therapeutic drug monitoring (TDM) in children treated with indinavir to prevent suboptimal drug levels resulting in virological failure and to prevent toxicity caused by high drug levels. Research on the relation between drug levels of protease inhibitors and differences in metabolism by cytochrome P450 3A isoenzymes may elucidate the cause of these interindividual differences. This may in the future be helpful in the prediction of an optimal drug dose. The use of micro-arrays, which will enable the parallel analysis of large numbers of genetic polymorphisms in different metabolic pathways may in the future contribute towards better understanding and prediction of these differences in the metabolism of protease inhibitors.

In **chapter 6** we show that twice daily indinavir administered in combination with the protease inhibitor ritonavir results in higher AUC's and trough levels of indinavir through the inhibition of CYP450 3A metabolism by a low-dose of ritonavir (100 mg/m^2). This study forms the basis for future studies with simplified twice daily regimens in children. However, again the pharmacokinetics of indinavir and low-dose ritonavir differed widely between individual children underlining the necessity for TDM.

Although there are definite clinical benefits from TDM, many pediatricians fail to use it as a regular clinical tool mainly because of practical problems with sampling. There are at least five different sampling strategies that can be used to perform TDM: analysis of full drug exposure (AUC), through levels, both peak and through levels, population based pharmacokinetic approaches (concentration ratios as used in the Dutch Athena cohort) or sophisticated PK modelling. Although it seems acceptable to study trough levels when one is analyzing virological efficacy, it is important to realize that trough levels are not a reliable indicator for virological response rates due to the inaccurate recording of the time when the last medication was taken. Another problem is that the trough level is not always similar to the Cmin and there may also be variation between trough levels in the evening and morning. In addition, the lower quantitation range of assays can be less accurate than in the middle or the highest range. To check toxicity levels in children one needs to analyze the Cmax. The time to sample the Cmax will vary from 1-4 hours depending on the Tmax for individual drugs and individual pharmacokinetics. Mathematical modelling can be performed from a sample taken at a random time point with knowledge of the patient's exact dosing times for the past couple of days. From one sample one can derive the AUC, the Cmax, and Cmin and the clearance for the patient. The difficulty of this approach is that different models are needed for each combination of drugs and that different metabolic rates are encountered at different ages. A large amount of data is

required to validate these models. It may be possible to generate reliable results by modeling of a simplified curve using drug levels at three time-points. Currently the only way to bypass these problems is to perform a full PK curve to determine the AUC and the other parameters such as Cmax and Cmin. This is a laborious, difficult and expensive procedure which contributes to the large expenses needed to perform appropriate care for HIV-1 infected children.

TDM is currently an inappropriate method for nucleoside reverse transcriptase inhibitors due to the difficulties in the measurement of intracellular phosphorylation. Nucleoside analogues are activated intracellularly by the addition of three phosphate groups. Measurement of the levels of triphosphorylated intracellular NRTI's is laborious and requires a large number of polymorphonuclear cells (and a large volume of blood), which makes this technique not useful in children. We recently developed a method using a Malditof massspectrometer to measure intracellular drug levels of nucleoside analogous' with only 12,500 cells (0.3 ml plasma). In addition, this method is very promising because it has the potency to become an automated process. With this technique it will be possible in the future to analyze the relation between intracellular levels of NRTI's and virological response rates.

In **chapter 7** we describe another application of TDM: the use of TDM to assess adherence to therapy. Adherence to therapy is required to obtain an optimal long-term virological response rate in HIV-1 infected children. An accurate assessment of the level of compliance is complicated by the limitation of the currently available methods. Plasma concentrations of protease inhibitors outside the limits of the reference values indicate non-adherence to antiretroviral therapy in adults. We observed that the compliance rates calculated as the percentage of samples that fulfilled the criteria for compliance using only the lower CORALs (concentration ratio limit consisting of the 5th percentile of population data obtained in adults) of indinavir or nelfinavir in children and the virological response rate are associated. This method may therefore be useful to assess non-compliance in children. However, it is based on population data obtained in adults. Population data obtained in children should be assembled to calculate concentration ratio limits in children. Since we observed a large interindividual variability in the pharmacokinetics of protease inhibitors, the use of concentration ratio limits consisting of the 5th percentile of population data obtained in children may not be the most optimal method. Optimization of this method may be possible by calculating CORALs in relation to the child's own PK curve. This will be analyzed and correlated to other adherence assessment strategies, which are currently used. These strategies include electronic pill boxes that registrate the dates and times a bottle was opened, and multidisciplinary support by specialized pediatricians, nurses, social workers and by the general practitioner and family home care. Adherence to therapy is supported by structured discussions with parents and children in which information on the importance of adherence to the therapy is given. In Belgium, recently an interactive cartoon on video with information on HIV infection, medication and adherence was developed for children of different ages. This video will be used during the routine visits to the nurse specialized in HIV/AIDS at our outpatient department. The focus of attention during these visits will be adherence to the antiretroviral therapy. To optimize the support structure for children with HIV/AIDS, we are currently analyzing the data obtained by a structured social background questionnaire. In this questionnaire the family's social network including, friends and other care-

givers is analyzed. The frequency and quality of support, the level of education, employment and the financial situation are explored. This questionnaire will be used in the future to prospectively, analyze risk factors for non-compliance with antiretroviral therapy.

Since the introduction of protease inhibitors in 1996, combination therapies including two reverse transcriptase inhibitors and a protease inhibitor have rapidly become standard therapy for HIV-1 infected adults. The data on efficacy of these combinations in children with HIV-1 infection are limited and are predominantly derived from studies with small numbers of children. In **chapters 9 and 10** the clinical, virological and immunological response rates in children with HIV/AIDS treated with HAART are studied with a follow-up period of 24 and 96 weeks respectively. The results of the treatment using PI's and NNRTI's have been excellent. Since the introduction of this therapy in 1997 only one child at a very advanced stage of disease in the initial phase of therapy died. Currently (January 2002), all other children are in an excellent clinical health. The two-year follow-up data indicate, that 69% of the children have a viral load below 500 copies/ml, and that 50% have a viral load below 40 copies/ml. These results are comparable with those obtained in adults. However, the majority of pediatric studies show inferior virological responses in comparison with those in adults. Several factors have been associated with a higher risk for virological failure. These include pharmacokinetic parameters (low plasma blood levels of protease inhibitors are associated with virological failure), inadequate adherence to antiretroviral therapy and differences in baseline characteristics (prior antiretroviral treatment, younger age, and high baseline viral load). It is striking that all four pediatric studies in which dosages of the administered PI were adjusted after pharmacokinetic evaluation report superior virological response rates compared with studies in which fixed dosages were used (**chapter 8**). The inferior virological response rates, which have been reported in HIV-1 infected children treated with HAART form a reflection of the challenges which are encountered in the treatment of children with HIV-1/AIDS. Difficulties with adherence and with the pharmacokinetics of protease inhibitors in children demand an intensive, child adjusted approach. Because of the necessity for treatment for an indefinite period, adherence will in the future increasingly become a problem. Optimizing therapy adherence by means of structured discussions with parents and children, using booklets in which stickers can be applied after dosing, and therapeutic drug monitoring requires increasing efforts from the multidisciplinary team. We recently started to use of electronic pill boxes in order to offer additional information on therapy adherence. Our social worker is analyzing the social backgrounds of the families of our children to identify social-economic factors that may interfere with adherence to therapy. This eventually may result in child and family-specific interventions. In addition, a cost-effectiveness analysis of this intensive intervention program will be performed.

Twice daily regimens and (in the future) once daily regimens of HAART may contribute towards a better therapy adherence. We are currently initiating new studies in which twice-daily therapy with two NRTI's and dual PIs versus a regimen with three NRTI's (abacavir, zidovudine and lamivudine) is used and in which adherence is measured with TDM, electronic pill boxes and structured interviews. The aims of future studies will include a lower pill burden, medication with less side-effects, improved support by a child-specific approach (for example by means of an interactive cartoon) and improved adherence by increased attention specifically directed to this issue by a

specialized nurse. In addition, the influence of therapy (swallowing pills, side-effects, stigmatising) on the quality of life is currently analyzed with the validated PEDsQoL questionaires.

In **chapter 11** the immunological responses to HAART are described. Since data on long-term T-cell dynamics in HIV-1 infected children on HAART were not available, it was still unclear to what extent the number of CD4+ T-cells of HIV-1 infected children could be normalized. CD4+ T-cell numbers in HIV-1 infected children on HAART recover more rapidly than CD4+ T-cells in HIV-1 infected adults. This observation has been attributed to the relatively large thymus and the better thymus function in young children. Our study is the first in which recovery of CD4+ T-cell counts is related to reference values for lymphocyte subpopulations. In general, absolute CD4+ T-cell counts are used in pediatric studies on T-cell repopulation during HAART, since CD4+ T-cells as percentages of total T-cell counts are influenced by the major changes in the number of CD8+ T-cells which are encountered in HIV-1 infected patients. However, analyses of T cell repopulation in groups of children with different ages are hampered by the fact that CD4+ T-cell counts are highly dependent on the age of the patients. Reference values in younger children are much higher and have a large range compared to those in older children. (15) Hence, younger children need to produce larger numbers of CD4+ T-cells to achieve their normal age-related CD4+ T-cell counts. The calculation of CD4+ T-cell counts as percentage of normal absolute values thus results in an independent parameter for the degree of CD4+ T-cell restoration. Using this method it appears from our data that CD4+ T-cell counts in older children are restored to the same degree relative to their normal values as in younger children. Even children with extremely low CD4+ T-cell counts at baseline did reach normal values during the 96 weeks of follow-up. Reconstitution of the immune system is predominantly caused by the production of naive CD4+ T-cells. In addition to the quantitative improvement of the immune system, the T-cell function also improved after stimulation with CD3 mAb plus CD28 mAb. This indicates that there is also functional improvement of T cells during HAART. Analyses on the changes of immune activation markers and the relation with virological response are ongoing. A remarkable finding was the absence of differences between virological responders and non-responders. This phenomenon could be explained by the selection of certain viral variants with resistance to protease inhibitors that have in-vitro impaired replicative capacity. This dissociation between continuing good clinical and immunological response rates in the presence of virological failure has been also observed by others. A longer follow-up and additional research are needed to better understand the underlying mechanisms of this phenomenon. The results from genotypical resistance analyses should be interpreted critically because of the frequent absence of clinical and immunological consequences. This is especially the case in heavily experienced patients who are left without treatment options.

Dysregulation of growth is a common feature of HIV-1 infection in children. Since growth seems to be one of the most sensitive indicators of disease progression in HIV-1 infected children, growth after the initiation of HAART is an important parameter in children. In **chapter 12** we describe the effects of therapy on growth in our cohort. We observed a positive effect of HAART on height and weight in children with HIV-1 infection. In contrast to studies with mono- and duo-therapy in which the positive effect of growth was only temporarily, this effect is sustained for at least 96 weeks and is associated with the successful application of HAART resulting in long-term viral load reduction and an increase of CD4+ T-cell counts. The etiology of the HIV-1 related growth dysregulation is

complex. Abnormal function of the thyroid gland, the somatomedine axis, the lipid metabolism and abnormal resting energy expenditure may also contribute to diminished growth. We therefore studied the endocrinological and immunological mechanisms underlying the recovery of growth in HIV-1 infected children treated with HAART.

In **chapter 13** we describe the results of this study. We determined cortisol, thyroid and growth hormones, and TNF-α serum levels. Hormones that influence growth postnatally include growth hormones and insulin-like growth factor I (IGF-I) as well as thyroid hormone and glucocorticoids. Excessive levels of glucocorticoids or very low levels of thyroid hormone during childhood result in growth retardation. These hormones play a permissive role in promoting growth stimulated by growth hormone and IGF-I. Tumor necrosis factor -α (TNF-α) is a pro-inflammatory and immunomodulatory cytokine with an increased production rate in the presence of infectious diseases and malignancies. TNF-α also strongly inhibits lipoprotein lipase and adipocyte gene expression. Chronically high levels of TNF-a have been associated with the development of cachexia. To determine changes in the growth hormone (GH) axis IGF-I and IGFBP-3 levels were measured. IGF-1 and its most important binding protein IGFBP-3 are known to be GH-dependent and have the major advantage that their concentrations are fairly constant throughout the day. Baseline levels of free T4 and cortisol were within the normal range and did not change during therapy, suggesting that hypothyroidism and adrenal axis abnormalities are not associated with recovery of growth after the initiation of therapy. Baseline serum levels of IGF-1 and IGFBP-3 are within the range of normal, but increased significantly after the initiation of therapy in this cohort with a high percentage of children with an optimal virological response (80%) and a significant decrease of TNF-α. The combination of relatively high serum levels of IGFBP-3 and low IGF-1 levels suggests the presence of a GH resistant state at baseline. The decrease in IGFBP-3 serum levels and the stabilization of IGF-1 levels after an initial significant increase suggests restoration of the sensitivity to GH and return to an anabolic condition during protease inhibitor containing therapy. Further research is needed to investigate whether other factors causing growth failure, such as inadequate caloric intake, abnormalities in lipid metabolism and total energy expenditure, may also play a role in the recovery of growth parameters during HAART and to confirm our hypothesis on the restoration of the sensitivity for GH.

Antiretroviral drugs such as indinavir need to be continued for many years thus underscoring the importance of a careful surveillance for long-term toxicity. The protease inhibitor indinavir may cause symptoms of nephrolithiasis through the formation of indinavir crystals. However, damage to the tubular epithelium may occur without symptoms. This sub-clinical phase can be followed by symptomatic renal injury. Symptomatic nephrotoxicity by the use of indinavir has been well described but little is known about the relevance of leucocyturia during the use of indinavir.

In **chapter 14** we present the first study to monitor nephrotoxicity in HIV-1 infected children on a prolonged treatment with indinavir. We observed a cumulative incidence of persistent sterile leucocyturia of 53% after 96 weeks (in children younger than 5.6 years: 78%) and a cumulative incidence of an increase in serum creatinine levels more than 50% above normal of 35% after 96 weeks. Children with persistent sterile leucocyturia more frequently had an increase in serum creatinine levels of more than 50% above normal. This suggests that persistent sterile leucocyturia is an early indication for the development of renal damage. We observed a higher

cumulative incidence of persistent sterile leucocyturia in children with an AUC of indinavir of > 19 mg/L*h and in children with a peak level of indinavir higher than 12 mg/L. Because of our previous observations which showed that an AUC of indinavir less than 20 mg/L*h was associated with virological failure, this complicates the treatment of HIV-1 infected children with indinavir. To achieve optimal virological suppression an AUC higher than 20 mg/L*h is required, but to avoid persistent leucocyturia an AUC less than 19 mg/L*h is needed. These observations suggest that indinavir may be less useful in the treatment of HIV-1 infected children. However, indinavir is a potent protease inhibitor, which in combination with nucleoside analogues gives an excellent long-term clinical, virological and immunological response in adults and in children as is showed by us in chapters 9 to 13. We therefore propose to monitor nephrotoxicity very closely and change therapy only in the case of overt signs of renal impairment. In this respect it is reassuring that the signs of renal impairment are reversible after discontinuation of indinavir. Since nephrotoxicity does not occur during the first 3 months of the therapy an alternative strategy could be an induction-maintenance regimen in which indinavir is used during the initial first months of therapy to strongly suppress virological replication followed by an easier, but less potent drug regimen.

In **chapter 15** we describe two cases of indinavir associated asymptomatic nephrolithiasis and cortex atrophy in HIV-1 infected children. Renal ultrasounds were performed because of persistent sterile leucocyturia in five children. In two children extensive calcifications and severe cortical atrophy were detected on ultrasound despite the absence of an increase in serum creatinine. We observed that these abnormalities were reversible after discontinuation of indinavir. Nevertheless we cannot exclude that this would also be the case in a more advanced stage of renal damage. In these two children a high AUC and Cmax of indinavir were observed. Since elevated plasma concentrations of indinavir have been associated with urological complications, the high AUC and Cmax may thus have contributed to the nephrotoxicity in these children. This observation underscores the need for routinely performed urinalysis and therapeutic drug monitoring in children treated with indinavir. In addition, we propose to regularly perform renal ultrasounds in children who are treated with indinavir and woh have an abnormal urinalysis.

A syndrome of hyperlipidemia, insulin resistance and peripheral lipodystrophy has been reported in HIV-1 infected adults since the registration of protease inhibitors in 1996. The prevalence of this syndrome is still unknown since many investigators only studied the prevalence of one aspect of the syndrome, because the three major symptoms (hyperlipidemia, insulin resistance and peripheral lipodystrophy) do not coexist in all patients and can be encountered in various combinations. An important factor in the analysis of the prevalence of lipodystrophy is the timing of the syndrome in relation to the start of antiretroviral therapy. Studies with a longer follow-up report a higher prevalence than those with a shorter follow-up. To date it has not been revealed whether these metabolic changes are the result of HAART of if HIV-1 infection itself is responsible. **Chapter 16** contains a study on changes in serum lipid levels and glucose-metabolism. Serum lipid levels of total cholesterol, HDL and LDL were significantly increased after 18 months or more of HAART. Serum glucose, insulin and C-peptide levels were normal. Hypertriglyceridemia and low levels of total cholesterol, HDL and LDL have been detected in HIV-1 infected patients without prior antiretroviral therapy, especially in the late phase of the disease. Therefore, one would expect a more significant rise in levels of total cholesterol, HDL and LDL in patients with an advanced stage of disease, who

are successfully treated with HAART as compared to children in an early phase of HIV infection. Thus, the significant rise in the serum levels of total cholesterol, HDL and LDL may not only be attributed to the effects of HAART, but may also partially be the result of a normalization of pre-existing lipid abnormalities. While among adults high incidences of clinically overt lipodystrophy are reported, we did not observe overt clinical symptoms of peripheral lipodystrophy and/or central obesity in our study. The absence of clinical signs of lipodystrophy should be further supported by analyses of objective standardized measurements, such as the waist/hip ratio's, the skinfold thickness measurements and dual-energy X-ray absorptiometry. Child-specific factors, like the presence of growth hormones or the absence of sex hormones may reduce the risk for peripheral lipodystrophy in children. Future research should focus on this issue, since it may contribute to the elucidation of the mechanism causing lipodystrophy in adults. In addition, differences in lipid metabolism between different protease inhibitor containing and protease inhibitor sparing treatment regimens should be investigated. In order to be able to perform this kind of studies large numbers of children are needed. Therefore these analyses should be performed in an international multicenter trial, for example in the European Paediatric European Network for Treatment of AIDS (PENTA).

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Samenvatting en discussie

Chapter 18

In **hoofdstuk 1** worden de achtergronden en de initiële doelstellingen beschreven van de studies, die in dit proefschrift gepresenteerd worden. In 1997 bundelden de drie academische kinderziekenhuizen (in Amsterdam, Rotterdam en Utrecht) die geconfronteerd werden met een toenemend aantal kinderen met HIV/aids hun krachten om te komen tot een optimalisering van de behandeling en de zorg voor deze kinderen. Als onderdeel van dit initiatief werd een multidisciplinaire werkgroep gevormd. Tegelijkertijd werd gestart met een gezamenlijk studieprotocol in alle academische centra in Nederland. Het doel van deze studie was het evalueren van het klinische effect, het virologische effect, de farmacokinetische en farmacodynamische aspecten van geselecteerde antiretrovirale middelen en de mate van herstel van het immuunsysteem volgend op het instellen van een combinatiebehandeling bestaande uit indinavir, zidovudine en lamivudine. Er werd een team van experts gevormd op immunologisch, virologisch en farmacologisch gebied en de terreinen van de interne geneeskunde en de kindergeneeskunde om nieuwe inzichten op het gebied van zorg, therapie en onderzoek bij volwassenen snel te kunnen toepassen en evalueren bij kinderen. Tegelijkertijd werd in het Sophia Kinderziekenhuis in Rotterdam een multidisciplinair team gevormd waarbij als taak gesteld werd de zorg voor kinderen met HIV/aids te optimaliseren. Dit team bestaat uit een gespecialiseerde verpleegkundige, een maatschappelijk werker, arts-onderzoekers en kinderarts-infectiologen. Het belangrijkste principe bij de opzet van de landelijke studie was dat de zorg voor met HIV geïnfecteerde kinderen georganiseerd zou moeten worden op een geprotocolleerde (wetenschappelijke) manier en dat onderzoeksprojecten dienstbaar moeten zijn aan de verbetering van de kwaliteit van leven van kinderen met HIV/aids.

In **hoofdstuk 2** wordt een overzicht gegeven van de inhoud van dit proefschrift, dat bestaat uit een serie studies op het gebied van de epidemiologie van HIV-1-infectie in Nederland, de effectiviteit van de preventie van de overdracht van HIV van moeder op kind, de farmacokinetische en farmacodynamische aspecten van krachtige antiretrovirale combinatietherapie bij kinderen, de effecten van deze therapie op het welbevinden en de groei bij met HIV geïnfecteerde kinderen, geselecteerde aspecten van immuunreconstitutie als gevolg van krachtige antiretrovirale combinatietherapie en een prospectieve analyse van de bijwerkingen van deze therapie.

In 1995 werd in Nederland gestart met de prospectieve registratie van alle kinderen die pre- of perinataal aan HIV geëxposeerd waren of postnataal gediagnosticeerd waren met een infectie door HIV. Kinderartsen meldden nieuwe kinderen elke maand aan het Nederlands Signalerings-Centrum Kindergeneeskunde. In **hoofdstuk 3** worden de effecten gepresenteerd van de implementatie van de richtlijnen ter preventie van perinatale transmissie van HIV in Nederland gedurende de periode 1995 tot 2000. Er werd een stijging waargenomen van het aantal kinderen dat perinataal aan HIV geëxposeerd was. Het percentage met HIV geïnfecteerde kinderen na perinatale expositie daalde in de periode 1995-2000 van 20% tot 4%. Deze verbetering vormt een reflectie van de toegenomen kennis van de pathogenese en behandeling van HIV infecties en van de wijze van transmissie. Het risico van perinatale overdracht van moeder op kind, dat zonder interventies 15-30% bedraagt (1-4), kan gereduceerd worden tot minder dan 2% door de behandeling van de moeder met krachtige antiretrovirale combinatietherapie en door de profylactische behandeling met zidovudine van het kind gedurende de eerste levensweken. Als de maternale hoeveelheid virus ten tijde van de partus in het bloed boven de 1000 kopieën/ml is, wordt een electieve sectio caesarea geadviseerd. De combinatie van een electieve sectio caesarea en antiretrovirale profylaxe aan het kind reduceert het

risico op transmissie van HIV tot bijna 0%. (5-9) De daling van het percentage door perinatale transmissie geïnfecteerde kinderen onder de aan HIV geëxposeerde kinderen van 20% tot 4% kan deels verklaard worden door de toename van het gebruik van krachtige antiretrovirale combinatietherapie door met HIV geïnfecteerde zwangere vrouwen. Dit gebruik steeg in dezelfde periode van 0% in 1995 tot 70% in 1999. Wij concluderen dat de Nederlandse richtlijnen voor de preventie van de overdracht van HIV van moeder op kind goed worden toegepast en dat deze richtlijnen effectief zijn.

In **hoofdstuk 4** worden gegevens gepresenteerd over het cumulatieve aantal kinderen in Nederland dat met HIV gediagnosticeerd werd, de wijze van transmissie, de risicofactoren en de klinische toestand vanaf de start van de aidsepidemie in de jaren '80 van de vorige eeuw tot het jaar 2000. De stijging van het aantal nieuw met HIV geïnfecteerde kinderen gedurende de periode 1995-1997 werd niet verder doorgezet tijdens de periode 1998-2000. Dit zou veroorzaakt kunnen zijn door een actievere benadering in de advisering en screening naar HIV bij zwangere vrouwen. Sinds 1999 wordt aan vrouwen met een verhoogd risico op HIV (vrouwen afkomstig uit een gebied met een gegeneraliseerde HIV- epidemie, vrouwen met sexueel contact met een man met een verhoogd risico op HIV) geadviseerd een HIV-test te ondergaan. Bij 86% van de met HIV gediagnosticeerde kinderen was één of beide ouders afkomstig uit een gebied met een gegeneraliseerde HIV-epidemie. De meeste kinderen (81%) waren geïnfecteerd door overdracht van moeder op kind. Vierentachtig procent werd in Nederland geboren. Het risico op transmissie van HIV had aanzienlijk gereduceerd kunnen worden wanneer de moeder voor de geboorte getest was op HIV en bij een bewezen HIV-infectie volgens de richtlijnen behandeld was. In 2000 hebben Postma en co-auteurs een kosteneffectiviteitsstudie verricht naar universele screening van HIV onder alle zwangere vrouwen in Amsterdam. (10) In deze studie werd aangetoond, dat een universele screening in Amsterdam zeer waarschijnlijk kosteneffectief is. Een probleem bij de analyse was dat er onvoldoende gegevens beschikbaar waren met betrekking tot de kosten van de behandeling van met HIV geïnfecteerde kinderen. Dit maakte een betrouwbare schatting van de kosten-baten-analyse onmogelijk. Wij zijn daarom recent in samenwerking met Postma gestart met een studie om de kosten van de behandeling van met HIV geïnfecteerde kinderen te analyseren.

Uit onze gegevens blijkt dat bij 86% van de kinderen één of beide ouders afkomstig is uit een gebied met een gegeneraliseerde HIV-epidemie. Het lijkt daarom onnodig om alle zwangere vrouwen in Nederland te screenen, zoals recent door de minister van Volksgezondheid, Welzijn en Sport werd geadviseerd. Een screeningsprogramma onder zwangere vrouwen die tot risiscogroepen behoren is naar onze mening een oplossing die veel kosten-effectiever is. Eén of beide ouders van 95% van de nieuwe met HIV gediagnosiceerde kinderen is afkomstig uit een ander land dan Nederland. De behandeling van deze kinderen wordt bemoeilijkt door problemen in de communicatie en door grote verschillen in sociaal, cultureel en economisch opzicht tussen de gezinnen van deze kinderen en de autochtone Nederlandse populatie. Deze situatie maken een zeer intensieve en gezins-specifieke benadering met aandacht voor de transculturele aspecten noodzakelijk. Het is duidelijk dat een dergelijke leidt tot veel hogere kosten in de zorg voor met HIV geïnfecteerde kinderen dan in de zorg voor volwassenen met HIV/aids.

De farmacokinetische en farmacodynamische aspecten van veel geneesmiddelen vertonen grote verschillen tussen kinderen en volwassenen. Het is daarom niet vanzelfsprekend dat een succesvolle

behandeling met geneesmiddelen bij volwassenen, bij kinderen tot hetzelfde resultaat leidt. De toepassing van nieuwe middelen bij kinderen, zoals de HIV-1-protease remmers, zou derhalve vooraf gegaan moeten worden door een gedetailleerde evaluatie van de farmacokinetische en farmacodynamische aspecten. Het doel van deze benadering is het bereiken van een gelijke effectiviteit en een gelijke of geringere toxiciteit in vergelijking met die bij volwassenen.

In **hoofdstuk 5** wordt de farmacokinetiek beschreven van de protease-remmer indinavir bij kinderen. Waarbij aanzienlijke interindividuele verschillen gevonden worden in de verschillende farmacokinetische parameters, zoals "area-under-the-time-curve concentration" (AUC), topspiegels en dalspiegels. De hogere metabole klaring en het grotere distributievolume van indinavir resulteren in suboptimale spiegels van indinavir bij veel HIV-1 geïnfecteerde kinderen. In een aantal eerdere studies bij volwassenen is een duidelijke relatie aangetoond tussen de concentratie van indinavir in het bloed en de virologische respons. (11-13) In hoofdstuk 5 wordt aangetoond, dat een AUC van indinavir hoger dan 20 mg/L*h geassocieerd is met een goede virologische respons. De grote leeftijdsafhankelijke individuele verschillen in de farmacokinetiek van indinavir, noodzaken tot routinematige bepaling van indinavir-spiegels in het bloed kort na de start van de behandeling. Bij te lage of te hoge spiegels dient een dosis aanpassing plaats te vinden. Aanvankelijk bestond de vrees dat het risico op aan indinavir gerelateerde nefrotoxiciteit (nierstenen, hematurie en flankpijn) bij kinderen hoger zou zijn dan bij volwassenen. Deze nefrotoxiciteit is het gevolg van precipitatie van indinavir-kristallen en is gecorreleerd aan hoge indinavir-topspiegels in plasma. (14) Hoewel de indinavir- topspiegels bij kinderen hoger zijn dan bij volwassenen, werden aanvankelijk bij geen van de kinderen nierstenen gevonden. Echter, in de hoofdstukken 14 en 15 is door middel van nauwkeuriger prospectief onderzoek naar de nefrotoxiciteit van indinavir bij kinderen gebleken dat een hoog percentage van de kinderen urologische bijwerkingen ondervindt van de behandeling met indinavir. Ook werd een relatie gevonden tussen de bloedspiegels van indinavir en de incidentie van nefrologische toxiciteit.

De onvoorspelbare interindividuele verschillen in de farmacokinetiek van protease-remmers (en waarschijnlijk ook van andere antiretrovirale middelen) bij kinderen onderstrepen het belang van het meten van medicatiespiegels in het bloed bij kinderen die behandeld worden met indinavir. Dit kan bijdragen aan het voorkomen van suboptimale medicatiespiegels met virologisch falen als gevolg daarvan, terwijl eveneens toxiciteit veroorzaakt door te hoge spiegels voorkomen kan worden. Onderzoek naar de relatie tussen medicatiespiegels van protease-remmers en verschillen in het metabolisme door cytochroom P450 3A isoenzymen, zal in de toekomst mogelijk leiden tot beter inzicht in de oorzaak van de interindividuele verschillen. Dit zou mogelijk kunnen leiden tot een à priori predictie van de optimale dosering van protease-remmers. De micro-arraytechniek, waarmee parallelle analyse van grote aantallen genetische polymorfismes in verschillende metabole systemen mogelijk is, zou in de toekomst kunnen bijdragen aan een beter begrip en predictie van de individuele verschillen in het metabolisme van protease-remmers.

In **hoofdstuk 6** wordt beschreven dat een twee-maal-daagse dosering van indinavir in combinatie met de protease-remmer ritonavir resulteert in hogere AUC's en dalspiegels door de inhibitie van het CYP450 3A metabolisme door een lage dosis ritonavir (100 mg/m^2). Deze studie vormt de basis voor toekomstige studies met een vereenvoudigde twee-maal-daagse dosering bij kinderen. De farmacokinetiek van indinavir in combinatie met een lage dosis ritonavir liet echter

opnieuw grote interindividuele verschillen zien. Eén en ander onderstreept opnieuw, die de noodzaak voor het routinematig bepalen van medicatiespiegels bij kinderen.

Hoewel de noodzaak voor het meten van medicatiespiegels in het voorgaande hoofdstuk duidelijk aangetoond is, worden spiegelbepalingen vanwege praktische problemen door nog slechts weinig kinderartsen/aidsbehandelaren toegepast. Er bestaan tenminste vijf verschillende strategieën voor het analyseren van medicatiespiegels: analyse van AUC; analyse van de dalspiegels; bepaling van zowel piek- als dalspiegels; op populatie gegevens gebaseerde farmacokinetische benaderingen (concentratieratio's zoals gebruikt in het Nederlandse Athena cohort) en geavanceerde berekeningen van de farmacokinetiek. Het meten van dalspiegels lijkt een acceptabele methode om de virologische effectiviteit te meten. Toch dient men zich ervan bewust te zijn dat dalspiegels niet altijd een betrouwbare maat zijn voor virologische effectiviteit zeker wanneer het tijdstip van laatste inname onjuist geregistreerd is. Een ander probleem is dat de dalspiegel niet altijd gelijk is aan de laagste concentratie en dat er een variatie bestaat tussen ochtend- en avonddalspiegels. Daarnaast kan de onderste detectiegrens van de meetmethode minder accuraat zijn dan het middelste en hoogste detectiegebied. De hoogste concentratie in het bloed (Cmax) dient bepaald te worden om van toxiciteit bij kinderen te voorkomen. De tijd waarop hiertoe na inname van het geneesmiddel bloed afgenomen moet worden, verschilt per middel van 1 tot 4 uur. Dit is afhankelijk van de, per middel verschillende, tijdsduur waarna de hoogste spiegel bereikt wordt en van individuele farmacokinetiek. Mathematische modellering kan toegepast worden op een willekeurig bloedmonster indien de exacte tijd van inname bekend is en een model op basis van populatiegegevens bestaat. Uit één bloedmonster kunnen door deze methode alle farmacokinetische parameters berekend worden. Het probleem van deze methode is dat verschillende modellen nodig zijn voor elke combinatie van geneesmiddelen en dat het metabolisme van de geneesmiddelen leeftijdsafhankelijk is. Hierdoor zijn grote hoeveelheden gegevens nodig om de modellen te valideren. Mogelijk kan men in de toekomst een vereenvoudigde farmacokinetiekcurve toepassen met een lager aantal bloedafnames. Op dit moment is de afname van een volledige farmacokinetiekcurve de enige mogelijkheid om een AUC, Cmax en Cmin te bepalen. Dit is een arbeidsintensieve, gecompliceerde en dure procedure, die mede leidt tot de hoge uitgaven die noodzakelijk zijn voor de zorg voor met HIV-1 geïnfecteerde kinderen.

Het bepalen van medicatiespiegels in het bloed is een ongeschikte methode ter bepaling van de effectiviteit van nucleoside reverse transcriptase-remmers (NRTI's). Deze NRTI's worden intracellulaire gefosforyleerd door de toevoeging van drie fosfaat-groepen. Het bepalen van de spiegels van getrifosforyleerde intracellulaire NRTI's is zeer arbeidsintensief. Bovendien is hiervoor een groot aantal polymorfonucleaire cellen (en een grote hoeveelheid bloed) nodig, hetgeen deze methode ongeschikt maakt voor gebruik bij kinderen. Recent is door ons een techniek ontwikkeld waarbij een Malditof-massaspectrometer gebruikt wordt voor het bepalen van intracellulaire spiegels van NRTI's met slechts 12.500 cellen (0,3 ml plasma). Daarnaast is deze methode veelbelovend vanwege de mogelijkheid tot automatisering ervan. Met behulp van deze techniek zal het in de toekomst mogelijk zijn de relatie tussen intracellulaire spiegels van NRTI's en de virologische effectiviteit te analyseren.

In **hoofdstuk 7** wordt een andere toepassing van het gebruik van medicatiespiegels beschreven: het meten van therapietrouw. Therapietrouw is noodzakelijk voor het bereiken van een optimale virologische respons bij met HIV-1 geïnfecteerde kinderen. Een adequate beoordeling van

de mate van therapietrouw wordt gecompliceerd door de beperkingen van de beschikbare methodes. Plasmaconcentraties van protease-remmers buiten de grenzen van referentiewaarden wijzen op een slechte therapietrouw bij volwassenen met betrekking tot de antiretrovirale behandeling. De mate van therapietrouw, berekend als percentage van de bloedmonsters, die voldeed aan de criteria voor therapietrouw, waarbij de laagste CORAL (concentratie ratio grens bestaande uit het 5e percentiel van populatiedata van volwassenen) van indinavir of nelfinavir gebruikt werd, was bij kinderen geassocieerd met de mate van virologische respons. Deze methode zou gebruikt kunnen worden bij de beoordeling van therapie-ontrouw bij kinderen. Zij is echter gebaseerd op populatiegegevens bij volwassenen. Het is in de toekomst noodzakelijk ook bij kinderen populatiedata te genereren om zo concentratieratiogrenzen te kunnen berekenen. Vanwege de grote interindividuele variabiliteit van de farmacokinetiek van protease-remmers bij kinderen, is het gebruik van concentratieratio-grenzen gebaseerd op populatiedata van kinderen waarschijnlijk niet de beste methode. De methode zou bij kinderen mogelijk verbeterd kunnen worden door het berekenen van CORAL's in relatie tot de individuele curve van elk kind. Dit zal geanalyseerd en gecorreleerd worden aan andere methoden voor het meten van therapietrouw die op dit moment gebruikt worden. Onder deze andere methoden vallen het gebruik van elektronische pillendozen die de datum en tijd registreren waarop de fles geopend is, en multidisciplinaire ondersteuning door gespecialiseerde verpleegkundigen, kinderartsen, maatschappelijk werkers, huisartsen en gezinszorg. Therapietrouw wordt ondersteund door gestructureerde discussies met ouders en kinderen waarin informatie wordt gegeven met betrekking tot het belang van therapietrouw. In België is een interactieve videoband gemaakt met informatie over HIV-infectie, medicatie en therapietrouw ten behoeve van kinderen op verschillende leeftijdsniveau's. Het gebruik van deze video zal geïmplementeerd worden in de bezoeken aan de gespecialiseerde verpleegkundige op de polikliniek. Tijdens deze bezoeken zal de nadruk gelegd worden op het verbeteren van therapietrouw. Om de gezinsondersteuning te verbeteren wordt op dit moment gebruik gemaakt van een gestructureerde sociale netwerkkaart. De resultaten van dit onderzoek zullen in de toekomst verder geanalyseerd en gevalideerd moeten worden. In de enquete wordt het sociale netwerk van een gezin, inclusief familie, vrienden en zorgverleners, geanalyseerd met betrekking tot de frequentie en kwaliteit van de ondersteuning, het opleidingsniveau van ouders, de arbeidssituatie en de financiële situatie.

Sinds de introductie van protease-remmers in 1996 is combinatietherapie met twee nucleoside reverse transcriptase-remmers en één proteaseremmer in snel tempo de standaardtherapie geworden bij volwassenen. De gegevens met betrekking tot de effectiviteit van deze combinaties bij HIV-1 geïnfecteerde kinderen zijn beperkt en zijn hoofdzakelijk tot stand gekomen door studies met een gering aantal kinderen. In de **hoofdstukken 9 en 10** worden de klinische, virologische en immunologische resultaten van de behandeling van met HIV-1-geïnfecteerde kinderen gedurende respectievelijk 24 en 96 weken geanalyseerd. De resultaten van de behandeling met protease-remmers en nucleoside reverse transcriptase-remmers zijn uitstekend. Sinds de introductie van deze therapie in 1997 is slechts één kind overleden. Dit kind was al in een zeer gevorderd stadium van de ziekte op het moment van de start van de therapie. Op dit moment (januari 2002) zijn alle andere kinderen in een uitstekende klinische conditie. De gegevens na behandeling gedurende twee jaar tonen dat 69% en 50% van de kinderen een onmeetbaar lage hoeveelheid virus in het bloed hebben

bij een detectiegrens van respectievelijk 500 en 40 kopieën/ml. Deze resultaten zijn vergelijkbaar met de resultaten die bij volwassenen beschreven worden. Een meerderheid van de studies naar de effectiviteit van antiretrovirale behandeling van met HIV-1 geïnfecteerde kinderen laat echter een inferieure virologische respons zien. Verschillende omstandigheden zijn geassocieerd met virologisch falen: farmacokinetische parameters (lage bloedspiegels van protease remmers), slechte therapietrouw en verschillende factoren voor aanvang van de therapie (voorbehandeling, lagere leeftijd, hoge hoeveelheid virus in het bloed). Het is opvallend dat alle vier pediatrische studies, waarin de dosering van de protease-remmers is aangepast, na evaluatie van farmacokinetische parameters een superieure virologische respons beschrijven ten opzichte van de studies waarin vaste doseringen toegepast werden **(hoofdstuk 8)**. De inferieure virologische resultaten bij kinderen die met krachtige antiretrovirale therapie behandeld worden, zijn een afspiegeling van de uitdagingen in de behandeling van met HIV-1 geïnfecteerde kinderen. Problemen met therapietrouw en met de farmacokinetische eigenschappen van protease-remmers bij kinderen eisen een intensieve, op kinderen toegespitste benadering. Vanwege de noodzaak van de behandeling gedurende een nog onbekende duur, zal therapietrouw in de toekomst toenemend een probleem gaan vormen. Verbetering van therapietrouw door gestructureerde gesprekken met ouders en kinderen, het gebruik van boekjes, waarin stickers geplakt kunnen worden na inname van de medicatie, en het meten van medicatiespiegels vereisen steeds meer inspanningen van het multidisciplinaire team. Recent werd gestart met het gebruik van elektronische pillendozen om tot aanvullende informatie te komen met betrekking tot therapietrouw. De sociale netwerkkaart wordt door de maatschappelijk werkster geanalyseerd om socio-economische factoren die met therapietrouw zouden kunnen interfereren op te sporen. Deze analyses moeten individueel en in relatie tot elkaar gevalideerd worden, hetgeen zou moeten leiden tot kind- en gezinsspecifieke interventies. Daarnaast zal een kosteneffectiviteitsanalyse van dit intensieve interventieprogramma uitgevoerd worden.

Twee-maal-daagse medicatieschema's en (in de toekomst) één-maal-daagse schema's zullen bijdragen aan een betere therapietrouw. Op dit moment wordt gewerkt aan nieuwe studies waarin een twee-maal-daagse therapie met twee NRTI's en een combinatie van indinavir en ritonavir vergeleken worden met een twee-maal-daags medicatieschema bestaande uit drie NRTI's (abacavir, zidovudine en lamivudine). In deze studie wordt therapietrouw gemeten met behulp van medicatiespiegels, elektronische pillendozen en gestructureerde interviews. Het doel van toekomstige studies zal zijn een geringere hoeveelheid pillen, een lagere innamefrequentie, minder bijwerkingen, verbeterde ondersteuning door een kind-specifieke benadering (bijvoorbeeld door de interactieve videoband) en verbeterde therapietrouw door meer aandacht voor therapietrouw door een gespecialiseerde verpleegkundige. Daarnaast zal de invloed van therapie (het slikken van pillen, bijwerkingen, stigmatisering) op de kwaliteit van leven geanalyseerd worden met de voor kinderen gevalideerde PEDsQoL-vragenlijst.

In **hoofdstuk 11** wordt de respons van het immuunsysteem op krachtige antiretrovirale combinatietherapie beschreven. Gegevens ten aanzien van de immunologische respons op therapie op langere termijn ontbraken waardoor het onduidelijk was in welke mate het aantal CD4+ T-cellen herstelde. Het aantal CD4+ T-cellen bij met HIV-1 geïnfecteerde kinderen herstelt sneller dan bij met HIV-1 geïnfecteerde volwassenen. Dit werd toegeschreven aan de relatieve grote thymus en de betere functie van de thymus bij jonge kinderen. Onze studie is de eerste waarin het herstel van het

aantal CD4+ T-cellen gerelateerd is aan de normaalwaarden voor lymfocytensubpopulaties. Over het algemeen worden in kinderstudies over T-cel repopulatie tijdens therapie absolute aantallen CD4+ T-cellen gebruikt, omdat het aantal CD4+ T-cellen als percentage van het totale aantal T-cellen beïnvloed wordt door grote veranderingen in het aantal CD8+ T-cellen tijdens therapie. De analyses van T-celrepopulatie in groepen kinderen met verschillende leeftijden worden beperkt doordat de normaalwaarden voor het aantal CD4+ T-cellen sterk leeftijdsafhankelijk zijn. Normaalwaarden bij jonge kinderen zijn veel hoger en hebben een groter bereik in vergelijking met oudere kinderen. (15) Jongere kinderen moeten daardoor grotere aantallen CD4+ T-cellen produceren om hun (leeftijdsafhankelijke) normale aantal CD4+ T-cellen te bereiken. De berekening van het aantal CD4+ T-cellen als percentage van normaalwaarden resulteert in een leeftijdsonafhankelijke parameter voor immuunrepopulatie. Op deze manier berekend, blijkt dat het aantal CD4+ T-cellen in relatie tot hun normaalwaarde bij jonge kinderen even goed herstelt als bij oudere kinderen. Zelfs kinderen met extreem lage aantallen CD4+ T-cellen bij aanvang van de therapie bereikten normale aantallen CD4+ T-cellen na 96 weken therapie. Immuunreconstitutie wordt hoofdzakelijk bereikt door de productie van naieve CD4+ T-cellen. Naast het kwantitatieve herstel van het immuunsysteem, wordt ook een herstel van de T-celfunctie na stimulatie met CD3 mAb plus CD28 mAb waargenomen. Dit suggereert dat ook een functioneel herstel optreedt na start van de therapie. Verder analyses met betrekking tot veranderingen in immuunactivatiemarkers en de relatie hiervan met virologische respons worden nog uitgevoerd. Een opmerkelijke bevinding was de afwezigheid van verschillen tussen kinderen met een goede virologische respons ten opzichte van degenen met een slechte virologische respons. Dit fenomeen zou verklaard kunnen worden door de selectie van bepaalde virusmutanten die resistent zijn tegen protease-remmers en die in-vitro een verminderde replicatiecapaciteit hebben. De dissociatie tussen een goede klinische en immunologische respons in de aanwezigheid van virologisch falen is ook waargenomen door anderen. Een langere follow-up duur en nader onderzoek zijn noodzakelijk voor een beter begrip ten aanzien van de onderliggende mechanismen van dit fenomeen. De resultaten van genotypische resistentieanalyse zouden kritisch geïnterpreteerd moeten worden vanwege de vaak afwezige klinische en immunologische consequenties. Dit geldt met name voor de uitgebreid voorbehandelde patiënten waarvoor weinig alternatieven overblijven.

Disregulatie van groei is een veel voorkomend verschijnsel van een HIV-1-infectie bij kinderen. Groei is één van de meest sensitieve indicatoren voor ziekteprogressie bij met HIV-1 geïnfecteerde kinderen. Herstel van groei na start van krachtige antiretrovirale combinatietherapie is derhalve een belangrijke parameter. In **hoofdstuk 12** worden de effecten van therapie op groei in ons cohort beschreven. Antiretrovirale therapie had een positief effect op lengte en gewicht. In tegenstelling tot studies met mono- en duotherapie waarin dit positieve effect op groei slechts tijdelijk gezien werd, werd herstel van groei waargenomen gedurende tenminste 96 weken. Herstel van groei is geassocieerd met een succesvolle toepassing van de therapie, met een langdurige suppressie van de virusreplicatie en met een stijging van het aantal CD4+ T-cellen. De etiologie van aan HIV-1 gerelateerde disregulatie van groei is complex. Een abnormale functie van de schildklier, afwijkingen in de groeihormoonas, het vetmetabolisme en een abnormaal energiemetabolisme zouden kunnen bijdragen aan een verminderde groei. Derhalve werd door ons een aantal endocrinologische en immunologische factoren geanalyseerd die ten grondslag zouden kunnen liggen aan het waargenomen herstel van de groei na therapie. In **hoofdstuk 13** worden de

resultaten van deze studie waarbij cortisol, schildklier- en groeihormonen en TNF-α spiegels in het bloed werden bepaald. Hormonen die de groei na de geboorte beïnvloeden zijn groeihormoon en insuline-achtige groeifactor-1 (IGF-1), schildklierhormoon en glucocorticocoïden. Excessieve glucocorticoïdspiegels of lage schildklierhormoonspiegels kunnen op de kinderleeftijd voor een achterblijvende groei zorgen. TNF-α is een pro-inflammatoir en immunomodulerend cytokine dat in verhoogde mate geproduceerd wordt bij infecties en maligniteiten. Daarnaast remt TNF-α lipoproteinelipase en de genexpressie van adipocyten. Chronisch hoge TNF-α- spiegels zijn geassocieerd met het ontwikkelen van cachexie. Om veranderingen in de groeihormoonas te analyseren werden IGF-1- en IGFBP-3-spiegels bepaald. Van IGF-1 en het belangrijkste IGF-1 bindende eiwit IGFBP-3 is bekend dat deze sterk afhankelijk zijn van groeihormoon. Deze hebben het belangrijke voordeel dat de concentraties constant zijn over de dag. De cortisol- en schildklierhormoonspiegels waren binnen de norm en veranderden niet na behandeling, hetgeen suggereert dat afwijkingen in schildklierhormoon en in de bijnieras geen rol spelen bij het herstel van de groei gedurende therapie. IGF-1 en IGFBP-3 spiegels in het bloed waren niet afwijkend maar stegen significant in het beloop van de behandeling. De TNF-α spiegels daalden significant tijdens de therapie. De combinatie van relatief hoge IGFBP-3-spiegels en lage IGF-1-spiegels suggereert dat er sprake is van groeihormoon resistentie. De daling van IGFBP-3-spiegels en stabilisering van IGF-1 spiegels wijzen in de richting van een herstel van de gevoeligheid voor groeihormoon een het intreden van een anabole conditie. Verder onderzoek is noodzakelijk om te onderzoeken of andere factoren die aan de disregulatie van groei gerelateerd zijn -calorische inname, vetmetabolisme en energiemetabolisme- ook een rol spelen bij het herstel van groei tijdens therapie en om deze hypothese ten aanzien van het herstel van de gevoeligheid voor groeihormoon te bevestigen.

Antiretrovirale geneesmiddelen zoals indinavir dienen gedurende vele jaren gecontinueerd te worden. Een zorgvuldige surveillance ten aanzien van bijwerkingen op de lange termijn is derhalve noodzakelijk. De protease-remmer indinavir kan klachten van nierstenen veroorzaken door de formatie van indinavir kristallen. Daarnaast kan schade aan het epitheel in de tubulus ontstaan zonder klinische symptomen. Deze subklinische fase kan gevolgd worden door symptomatische nierschade. Symptomatische nefrotoxiciteit door het gebruik van indinavir is in de literatuur bij volwassenen goed gedocumenteerd. Over de relevantie van leucocyturie gedurende het gebruik van indinavir is echter weinig bekend. In **hoofdstuk 14** wordt de eerste prospectieve studie beschreven naar nefrotoxiciteit bij met HIV-1 geïnfecteerde kinderen, die langdurig met indinavir behandeld werden is. De cumulatieve incidentie van persisterende steriele leucocyturie bedroeg 53% na 96 weken therapie met indinavir. Bij kinderen jonger dan de mediaan van 5,6 jaar was de cumulatieve incidentie 78%. Daarnaast werd bij 35% van de kinderen na 96 weken een cumulatieve incidentie van een stijging van het serum-kreatinine van meer dan 50% boven de leeftijdsafhankelijke normaalwaarde gevonden. Kinderen met een persisterende steriele leucocyturie hadden vaker een stijging in het serum-kreatinine van meer dan 50% boven normaal. Dit suggereert dat persisterende steriele leucocyturie een vroege indicator is voor het ontwikkelen van nierschade. Een hogere cumulatieve incidentie van persisterende steriele leucocyturie werd gevonden bij kinderen met een AUC van indinavir > 19 (mg/L*h) en bij kinderen met een topspiegel van indinavir hoger dan 12 (mg/L). Dit compliceert de behandeling van met HIV-1 geïnfecteerde kinderen met indinavir, omdat wij eerder hebben beschreven dat een AUC van indinavir < 20 (mg/L*h) geassocieerd is met

virologisch falen. Om optimale virologische suppressie van HIV te bereiken is een AUC > 20 (mg/L*h) nodig, maar om het risico op nefrotoxiciteit te verkleinen is een AUC < 19 (mg/L*h) nodig. Deze observaties suggereren dat indinavir minder bruikbaar is bij de behandeling van met HIV-1 geïnfecteerde kinderen. Indinavir is echter een zeer krachtige protease-remmer, die in combinatie met nucleoside-analoga een uitstekende lange termijn klinische, virologische en immunologische response bewerkstelligt bij zowel volwassenen als bij kinderen zoals gedemonstreerd is in de hoofdstukken 9 tot en met 13. Gesteld wordt derhalve voor nefrotoxiciteit zorgvuldig te monitoren en indinavir alleen te vervangen indien een verslechtering van de nierfunctie optreedt. Dit is ons inziens zonder risico's, omdat de gerapporteerde nierschade reversibel is na het staken van de behandeling met indinavir. Nefrotoxiciteit komt nauwelijks voor gedurende de eerste drie maanden na start van de behandeling. Een alternatieve strategie zou derhalve kunnen bestaan uit een inductie-onderhoudsschema, waarbij indinavir gedurende de eerste maanden van de behandeling gebruikt wordt om de virusreplicatie krachtig te onderdrukken, gevolgd door een eenvoudigere en minder toxische, maar ook minder krachtige combinatie.

In **hoofdstuk 15** worden twee ziektegeschiedenissen beschreven van door indinavir veroorzaakte asymptomatische nefrolithiasis en cortexatrofie bij met HIV-1 geïnfecteerde kinderen. Een echografisch onderzoek van de nieren werd bij vijf kinderen verricht in verband met het bestaan van persisterende steriele leucocyturie. Bij twee kinderen werden uitgebreide calcificaties en ernstige atrofie van de cortex waargenomen op de echo ondanks de afwezigheid van een stijging van het serum kreatinine. Deze afwijkingen waren reversibel na staken van indinavir. Desondanks kan niet uitgesloten worden dat bij een verder gevorderd stadium van nierschade een blijvende reductie van de nierfunctie kan optreden. Bij deze twee kinderen werd een hoge AUC en Cmax van indinavir gemeten. De hoge bloedspiegels kunnen hebben bijgedragen aan het ontstaan van nierschade. De door ons gerapporteerde observatie onderstreept de noodzaak voor het routinematig verrichten van urine-onderzoek en het meten van serumspiegels van indinavir.

Een syndroom bestaande uit hyperlipidemie, insulineresistentie en perifere lipodystrofie is reeds sinds de registratie van protease-remmers in 1996 beschreven bij met HIV-1 geïnfecteerde volwassenen. De prevalentie van dit syndroom is nog steeds onbekend doordat veel onderzoekers slechts één aspect van het syndroom onderzochten en doordat de drie hoofdsymptomen (hyperlipidemie, insulineresistentie en perifere lipodystrofie) niet altijd samen voorkomen bij alle patiënten. Een belangrijke factor in de analyse van de prevalentie van lipodystrofie is het tijdstip van optreden na start van de behandeling met protease-remmers. In studies met een langere follow-up-tijd wordt een hogere prevalentie vastgesteld dan in studies met een kortere follow-up-tijd. Het is nog steeds niet opgehelderd of de metabole veranderingen het gevolg zijn van de therapie of van de HIV-1-infectie zelf.

In **Hoofdstuk 16** wordt een studie gepresenteerd naar de veranderingen in serumspiegels van lipide- en glucosemetabolisme. Totaal cholesterol, HDL- en LDL-cholesterol in serum stegen significant na toediening van krachtige antiretrovirale combinatietherapie gedurende tenminste 18 maanden. De serumspiegels van glucose, insuline en C-peptide waren normaal. Hypertriglyceridemie en lage serumspiegels voor totaal cholesterol, HDL en LDL zijn eerder beschreven bij met HIV-1 geïnfecteerde patiënten, met name bij patiënten in een verder gevorderd stadium van de ziekte. Een sterkere stijging van het totale, HDL- en LDL-cholesterol zou derhalve verwacht kunnen worden bij kinderen in een verder gevorderd stadium van de ziekte na start van de behandeling in vergelijking

met kinderen in een minder gevorderd stadium. De significante stijging van het serum totaal, HDL- en LDL-cholesterol zou dus ook het resultaat kunnen zijn van een normalisatie van pre-existente afwijkingen in het lipidenspectrum. Hoewel bij volwassenen hoge incidenties van klinisch zichtbare lipodystrofie gemeld worden, zijn bij de kinderen in de studie nog geen zichtbare symptomen van lipodystrofie en/of centrale obesitas waargenomen. De afwezigheid van klinische aanwijzingen voor lipodystrofie dient ondersteund te worden door de analyse van objectieve gestandaardiseerde metingen, zoals middel-heup-ratio's, huidplooimetingen en "dual energy X-ray absorptiometry". De aan- of afwezigheid van specifieke factoren bij kinderen, zoals de aanwezigheid van groeihormoon of de afwezigheid van geslachtshormonen, zou het risico op het ontstaan van perifere lipodystrofie bij kinderen kunnen verminderen. Toekomstig onderzoek zou zich moeten richten op het ophelderen van het mechanisme dat ten grondslag ligt aan lipodystrofie bij volwassenen. Daarnaast dienen verschillen in het vetmetabolisme tussen verschillende behandelingsstrategieën vergeleken te worden. Om dit soort studies te kunnen verrichten zijn grote aantallen kinderen nodig. Deze analyses zouden kunnen plaatsvinden in internationaal verband, bijvoorbeeld in het " Paediatric EuropeanNetwork for Treatment of AIDS" (PENTA).

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Conclusions and future perspectives

Chapter 19

In this chapter we briefly summarize our conclusions and we present our ideas concerning the future directions of care and research in children with HIV/AIDS. In Table 1 the major conclusions and the future directions for research are summarized.

During the past four years a multidisciplinary team became responsible for the care of children with HIV/AIDS in the Sophia Children's Hospital in Rotterdam. This team includes nurse specialists, a social worker, research physicians and pediatric infectious disease experts. In these years we carefully delineated the responsibilities of the different teammembers. The nurse specialist focuses on adherence, providing information on the disease and its treatment and making home visits. The social worker organizes contacts with organizations outside the hospital, collects information on social background (with the social background questionnaire) and provides support in economical and family problems. The research physician's responsibility includes the organization of a structured approach, correct data collection, the writing of study protocols, and the analysis and publication of data. The pediatric infectious disease experts provide state-of-the-art medical care and supervision of all activities in collaboration with the other members of the team. Central point of attention is optimization of adherence to therapy. This starts with the assessment of therapy by structured discussions with parents and children, by the use of electronic pill boxes that registrate the dates and times a bottle was opened, and by therapeutic drug monitoring. Adherence to therapy is further enhanced by providing information on the importance of this aspect of the therapy. In Belgium, recently an interactive cartoon on video with information on HIV infection, medication and adherence has been developed for use in children of different ages. With financial support of the Sophia Foundation in Rotterdam, we will start to use this interactive computer program to further optimize the care for and the compliance with therapy in our children. To obtain a better understanding of social and economic factors contributing towards adherence to therapy, we are currently analyzing data derived from a social background questionnaire. Since adherence to medication often decreases over time, further improvements in the level of care will be necessary to maintain optimal long-term treatment results. An individualized and flexible approach towards treatment regimen will be essential to optimize adherence to HAART.

Since the social situation of a family may have a tremendous impact on the ability to consistently access care and adhere to medication regimens, a family outpatient department should be established in which both parents and children are seen by doctors, nurses and social workers. In this respect the absence of financial resources for a psychologist as a member of the multidisciplinary team restricts our opportunities to provide optimal care. Increased understanding of the family's social situation will result in the possibility to apply individualized interventions. Improved communication with local pediatricians and the family's general practitioners resulting in involvement and participation in the treatment of the families will further optimize long-term treatment. Currently protocols for improved communication and collaboration between all parties involved are developed. An emerging problem is the increasing number of children reaching adolescence resulting in adolescent specific adherence problems (denial of disease, rebellious behavior). Special attention, support and adolescent talk groups may be able to help these children through their puberty specific problems. However, experiences and different strategies should be shared and monitored in a structured way. Because of the limited numbers of adolescents in one country, this should be performed in a national or maybe even international context. For younger children that are dependent on their parents or caregivers for an adequate treatment, adherence

problems should be followed-up very carefully. The development of protocols on strategies of interventions taken in the case of non-adherence in collaboration with Child Welfare Council, home-care organizations, jurists and judiciary should warrant optimal care for children with incapable or unwilling parents.

Finally, we have shown in this thesis that the treatment of HIV-1 infected children under the conditions as mentioned above can be as successful as that in adults. This will lead to an optimal clinical response (no opportunistic infections, recovery of growth), optimal virological suppression, a maximal recovery of the immunesystem and generally mild and transient side-effects of the antiretroviral drugs.

	Major conclusions	Future directions for research
Epidemiology	• In the period from 1995-2000 the percentage of HIV-1 infected children after exposure to HIV-1 decreased from 20% to 4%, suggesting that the Dutch guidelines to prevent mother-to-child prevention are well implemented and efficacious.	• Continuing registration to observe whether this percentage may be further decreased by the administration of HAART to pregnant women who are infected by HIV and to monitor possible toxicity associated with prenatal exposure to antiretroviral medication.
	• The increase in the number of HIV-1 infected children during the period 1995-1997 reached a plateau in the period 1998-2000. • Eighty-six percent of the children originated from a country with a generalized HIV epidemic. • Eighty-one percent of the children was infected by mother-to-child transmission.	• Cost-benefit analysis of universal screening on HIV in pregnant women versus a more focussed program to test of pregnant women belonging to high risk groups. • Analyses of the costs of the care and treatment for children with HIV/AIDS.
Pharmacology	• The unpredictable interindividual differences in the pharmacokinetics of protease inhibitors in children underscore the importance of TDM in children treated with indinavir to prevent suboptimal drug levels resulting in virological failure and to prevent toxicity caused by high drug levels.	• Monitoring of indinavir plasma levels shortly after the start of indinavir in stead of using a fixed dose regimen. • The development of a simplified pharmacokinetic curve using drug levels at less time-points and yet still obtaining reliable estimates for pharmacokinetic parameters. • Future research on the relation between drug levels of protease inhibitors and differences in metabolism by cytochrome P450 3A isoenzymes may elucidate the cause of these interindividual differences.
	• Twice daily indinavir administered in combination with ritonavir results in higher AUC's and trough levels of indinavir by the inhibition of CYP450 3A metabolism by low-dose ritonavir. • Again the pharmacokinetics of indinavir and low-dose ritonavir differed widely between individual children underscoring the necessity to perform TDM.	• Study of the clinical, virological and immunological effectiveness of an indinavir/ritonavir containing regimen. • Development of simplified regimens including less frequent dosing of NRTI's. • Intracellular measurement of druglevels will be necessary to evaluate whether adequate druglevels of NRTI's are preserved. • A novel technique requiring a little volume of blood is currently developed by our group using a Malditof Massspectrometer.
	• Therapeutic drug monitoring in relation to concentration ratio limits consisting of the 5th percentile of population data obtained in adults may be useful to assess non-adherence in children.	• Population data obtained in children should be generated to calculate concentration ratio limits in children. • Optimization of this method may be possible by calculating CORALs in relation to the child's own PK curve. This will be analyzed and correlated to other adherence assessment strategies, which are currently used.

	Major conclusions	Future directions and research
Clinical, virological and immunological aspects	• No opportunistic infections were observed. • Virological response rates (69% <500 copies/ml, 50% <40 copies/ml) after 96 weeks are comparable to those in adults. • Poor compliance is associated with poor virological respons.	• Studies with twice (or in the future once)-daily regimens with more active protease inhibitors, with lower pill burden, and less side-effects, which will result in improved adherence. • Improved support by a child specific approach. • Study on the quality of life with the PEDs QoL questionnaire and validation of this questionnaire. • Improvement of compliance by improvement of support (psychologist, social background analysis, interactive computer program, adolescent talk groups), lower pill burden, less side-effects.
	• Increase in height and weight (expressed as standard deviation scores) associated with successful application of HAART. This is associated with an increase of IGF-1, but not with cortisol, thyroid hormone and TNF-α changes.	• Study with a larger number of children to investigate the underlying principles of the recovery of growth after the initiation of HAART (including caloric intake, lipid metabolism and resting expenditure)
	• Normalization of CD4+ T-cell counts (mainly by an increase of naive CD4+ T-cells) in children of all age groups. Even in children with extremely low CD4+ T-cell counts at baseline. • Differences in immunological response between virological responders and non-responders were not observed. • Functional improvement of the immune system (stimulation with CD3 mAb plus CD28 mAb).	• Studies on other aspects of immunoreconstitution: cytotoxic T-cell responses, cytokine profiles and activation markers. • Studies on the relation between genotypic resistance and clinical and immunological response rates.

	Major conclusions	Future directions and research
Side-effects	• In children treated with indinavir 53% experienced persistent leucocyturia after 96 weeks associated with loss of renal function. • Persistent leucocyturia was more frequent in children <5.6 years, children with AUC >19 mg/L or Cmax >12 mg/L. • Increased total cholesterol, HDL and LDL cholesterol were observed after >18 months of HAART. This may be due to a normalization of pre-existing lipid abnormalities. • Glucose, insulin and C-peptide levels were normal. • No overt clinical symptoms of peripheral lipodystrophy and/or central obesity were observed.	• Close monitoring of nephrotoxicity and change of the therapy in the case of loss of renal function. • Since the cumulative incidence of persistent leucocyturia increases after 3 months, the concept of a highly active induction regimen (with indinavir) followed by an less toxic and simplified maintenance regimen should be explored. • Prospective study on changes in lipid metabolism, waist/hip ratio's and skin fold thickness. • Analysis of differences in biochemical and clinical parameters between different medication regimens. • Study of the underlying pathophysiological mechanisms that may clarify the difference in the prevalence of lipodystrophy between adults and children (i.e. the presence of growth hormone, the absence of sex hormones).

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Curriculum vitae

Annemarie van Rossum was born in Haarlem, The Netherlands, on April 4, 1973. She passed her secondary school exam (Gymnasium) in 1991 at the "Eerste Vrijzinnig Christelijk Lyceum" in The Hague. In 1991, she started her medical training at the Erasmus University Rotterdam. In 1995 she participated in a research project on the glucose metabolism in preterm neonates at the Department of Pediatrics, Division of Neonatology of the Sophia Children's Hospital in Rotterdam (head: Prof. dr. P.J.J. Sauer). In 1998 the Medical Degree was obtained and the research presented in this thesis in the Department of Pediatrics, Division of Infectious Diseases and Immunology (head: Prof. dr. R. de Groot) was started. This project involved several studies on clinical, virological and immunological aspects of treatment with highly active antiretroviral therapy in HIV-1 infected children. In addition, studies on the epidemiology of HIV-1 infected children in The Netherlands, pharmacokinetics and pharmacodynamics of protease inhibitors in children and on the side-effects of these antiretroviral drugs were initiated. During this period she was involved in two projects in Romania which aim to provide care and adequate treatment for children with HIV/AIDS ("Stichting bouwen voor zwakzinnige kinderen", Peatra Neamt and "Kinderen van de Zwarte Zee", Constantza, Romania). In September 2001 she enrolled in the residency program in Pediatrics at the Sophia Children's Hospital/Erasmus MC, Rotterdam, The Netherlands (head: Prof. dr. H.A. Büller).

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List of abbreviations

3TC	=	Lamivudine
ABC	=	Abacavir
AT	=	As treated analysis
AUC	=	Area under the Concentration versus Time Curve
BMI	=	Body mass index (kg/m2)
CDC	=	Centers for Disease Control and Prevention
Cmax	=	Peak level
Cmin	=	Trough level
d4T	=	Stavudine
DdC	=	Zalcitabine
DdI	=	Didanosine
EFV	=	Efavirenz
FDA	=	US Food and Drug administration
Free T4	=	Free fraction of circulating thyroxine
GH	=	Growth hormone
HAART	=	Highly active antiretroviral therapy
HIV-1	=	Human immunodeficiency virus type 1
IDV	=	Indinavir
IGFBP-3	=	Insulinlike growth factor binding protein 3
IGF-I	=	Insulinlike growth factor
IQR	=	Interquartile range
ITT	=	Intention to treat analysis
NFV	=	Nelfinavir
NNRTI	=	Non-nucleoside reverse transcriptase inhibitor
NRTI	=	Nucleoside reverse transcriptase inhibitor
NVP	=	Nevirapine
PI	=	Protease inhibitor
RTV	=	Ritonavir
SD	=	Standard deviation
SDS	=	Standard deviation score
SQV	=	Saquinavir
TDM	=	Therapeutic drug monitoring
TNF-α	=	Tumor necrosis factor alpha
ZDV	=	Zidovudine