Factors Associa h Clinical, Immunological and Virological Responses in Protease-In -Experienced Brazilian Children Receiving Highly Active roviral Therapy Containing Lopinavir-Ritonavir

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This study evaluates clinica gical and immunological responses to antiretroviral (ARV) therapy based on Lopinavir/ritonovir (LPV/r) ously protease -inhibitor-experienced children. The study included 29 Brazilian children (median age = 5.91 ho had failed previous ARV therapy and had begun a regimen based on LPV/r. At 12 months follow-up, a g gical response to LPV/r therapy was defined as achieving an undetectable viral load or as a decrease in plas RNA levels to ≥ 1 log. A good immunological response was defined as an increase in CD4$^+$ cell count from ba fficient to attain a better CDC immune stage classification. The number of infectious episodes 12 months before and 12 months after beginning LPV/r was assessed. Sixteen (55.2%) and 19 (65.5%) of 29 patients exhibited good virological and immunological responses, respectively. Baseline CD4$^+$ values (>500) predicted both virological and immunological responses (p<0.05). Older children were less likely to develop an immunological response (p<0.001) than younger children. Nine children receiving 3 ARV drugs plus LPV/r showed an immunological response (100%) compared to 10/20 (50%) children receiving 2 drugs plus LPV/r (p=0.01). A lower number (n<5) of infectious episodes was noted after 12 months follow-up in children using the LPV/r regimen (p=0.006). There was a positive correlation between children whose baseline CD4$^+$ values were greater than 500 cells/mm^3 and virological responses. Although virological responses to therapy were seen in about half the children (55.2%), the use of HAART containing LPV/r provided clinical and immmunological benefits.

Key-Words: HIV, children, HAART, lopinavir/ritonavir.

The use of highly active antiretroviral therapy (HAART) has greatly improved the prognosis of HIV-1-infected children [1-3]. However, in most pediatric studies the virological responses to HAART are less well developed than those in adults [4,5]. Poor adherence to therapy and low tolerability are frequently cited as culprits, as are prior treatment experience, the emergence of drug-resistent viruses, and suboptimal pharmacokinetic properties of the drugs [6,7]. In attempts to supplant some of these difficulties, therapeutic regimens have been modified by the use of boosted protease inhibitors (PIs) emploing ritonavir [8] or by the introdution of co-formulations. The first boosted PI was developed as a fixed-dose co-formulation of lopinavir/ritonavir (Kaletra)**.** Lopinavir, the active component of this combination, is extensively metabolized by the P450 cytochrome (CYP) system, and produces low systemic concentrations when used alone. Ritonavir potently inhibits CYP3A4 and is used to enhance systemic exposure to lopinavir [9].

Compared to other PIs, lopinavir/ritonavir may have advantages in terms of pharmacokinetics, efficacy, and resistance [7].

The aim of the present study was to evaluate factors associated with clinical, virological and immunological responses to antiretroviral (ARV) therapy based on LPV/r in protease-inhibitor-experienced children.

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Materials and Methods

Study

This longitudinal, single center, observational study was conducted at the Pediatric AIDS Outpatient Clinic of the Federal University of São Paulo, Brazil, from July 2001 to July 2004.

Patients

Twenty-nine children met the inclusion criteria, which consisted of having started highly active antiretroviral therapy containing LPV/r after failing one or more previous regimens, with a minimal follow-up period of 12 months.

Baseline characteristics of the study population are shown in Table 1. Although all children were nucleoside analogue and protease inhibitor experienced at abseline, ten children (34.5%) had failed previous therapies consisting of 2 nucleoside analogues plus a single non-nucleoside analogue (nevirapine or efavirenz). The reverse transcriptase inhibitor regimens consisted of estavudine and didanosine (12/29; 41.4%), zidovudine and didanosine (7/29, 24.1%), estavudine and lamivudine (4/29; 13.8%) or zidovudine and lamivudine (3/29,10.3%). Four children (13.8%) were using double therapy (estadudine plus didanosine or estavudine plus lamivudine) at the time they started HAART with LPV/r.

The protease inhibitors most frequently used in the failed schemes were ritonavir (6/29; 20.7%), nelfinavir (5/29; 17.2%) and amprenavir (4/29; 13.8%).

Laboratory Methods

Children underwent regular assessment of laboratory parameters at intervals ranging from 4 to 5 months during follow-up.

Plasma HIV-1 RNA was measured using a quantitative, reverse transcriptase-polymerase chain reaction with a lower detection limit of of 400 copies/mL (Roche Molecular Systems, Branhburg, New Jersey, USA). Peripheral blood mononuclear cells were assessed by flow cytometry (FACSCalibur, BD Biosciences, USA) immediately after cell staining, and were analyzed using CellQuest software (BD Biosciences, USA).

Statistical Analysis

All results are presented as medians and ranges. Virological responses were classified into three categories: group I included children with undetectable HIV-1 RNA values (< 400 copies/mL) at 12 months follow-up (complete virological responders); group II included children in whom plasma HIV-1 RNA did not decline to undetectable levels, but showed a sustained viral load drop ≥ 1 log at 12 months (partial virological responders); group III comprised children with treatment failures whose plasma HIV-1 RNA levels showed a non-sustained drop or a drop of less than 1 log at 12 months follow up (virological failures).

To evaluate immunological responses, children were reclassified at the last time of follow up (12 months) according to the CDC Classification System (1994)[10]. A good immunological response was considered present when the child's immune stage changed from 3 to 2 or 1, or from 2 to 1. Children who began the study classified as immune stage 1 and remained at this same level of classification at 12 months follow up, were also considered as having a good immunological response. Those who showed no immunological improvement based on the above criteria were considered to be immunological non-responders. Differences between these categories of predictor variable were evaluated using a univariate analysis of variance on ranked data. The Chi-squared test (χ^2) or Fisher exact test was used to compare groups. The threshold significance was set at p=0.05. Statistical analyses were performed using the Stata Software package version 7.0.

Clinical improvement was assessed by comparing the number of infectious episodes 12 months before and 12 months after the introduction of HAART with LPV/r (HAART-LPV/r).

Results

As shown in Table 1, the median baseline viral load values were similar among the three groups studied. The median $CD4^+$ counts ($mCD4^+$) were higher in group I (complete virologial responders; $mCD4^+$=1,533, range 121–2,680) than for groups 2 and 3 (partial virological responders; $mCD4^+$= 239, range 9–692; and virological failures; $mCD4^+$=201, range 7–1,198), although the differences were not statistically significant.

At the last time of follow up, 11 patients showed full complete virological responses (37.9%), 5 exhibited partial

Table 1. Baseline characteristics of children in the study population

	Complete virological responders	Partial virological responders	Virological failure	Total
N	11	5	13	29
Median age (years)	5.4	6.59	8.47	5.91
Clinical stage according to CDC* criteria				
Non-C	5	4	6	15
C	6	1	7	14
Immunological stage according to CDC* criteria				
Non-3	10	1	6	17
3	1	4	7	12
Median (IQ range) viral load (log_{10})	4.7 (3.2-5.6)	5.07 (3.0-5.8)	4.9 (2.7-5.7)	4.84 (3.0-5.7)
Median (IQ range) CD4 cell/mm^3	1,533 (121-2,680)	239 (9-692)	201 (7-1,198)	486 (7-2,690)
Therapeutic history				
Previous treatment without PI	6	2	5	13
Previous treatment with PI	5	3	8	16
Less than 3 prior ARV schemes	2	0	2	4
≥ 3 prior ARV schemes	9	5	11	25

* CDC, Centers for Disease Control and Prevention; IQ, interquatile range; PI, protease inhibitor.

Table 2. Type and frequency of infectious disease events in the study population

Infectious disease event	Episodes (n)	%
URI/ common cold	84	30.9
Tonsilitis	12	4.4
Otitis media, acute	25	9.2
Otitis media, chronic	22	8.1
Sinusitis	14	5.2
Pneumonia	30	11.0
Diarrhoea	15	5.5
Mucocutaneus infection	11	4.0
Others	49	18.0
Total	271	100

virological responses (17.2%) and 13 were virological failures (44.8%). Considering the complete responders and the partial responders as a single group, 16 (55.2%) children were considered to be virological responders. Nineteen children (65.5%) showed an increase in $CD4^+$ cell counts with improvement in CDC immune stage. Ten patients (34.5%) showed no immunological improvement after 12 months of HAART with LPV/r. The failed therapeutic schemes used prior to starting the study consisted of 2 NRTIs plus 1 PI (n=10), 2 NRTIs plus 1 NNRT (n= 10), 2 NRTI (n= 4), 1 NRTI plus 2 PIs (n=3), 3 NRTI plus 1PI (n=1), 1 NRTI plus 1 NNRTI plus 1 PI (n= 1). When considering the number of ARV drugs that comprised the LPV/r scheme, most children (n=20; 69%) received 2 ARV drugs plus LPV/r, while 9 (31%) received 3 drugs plus LPV/r.

Twenty-three (86.21%) patients were very ARV-experienced, having participated in 3 or more ARV schemes prior to the present study.

The frequency and kind of infectious disease episodes that occurred during the 12 months pre- and post-introduction to HAART with LPV/r are given in Table 2. The most frequent episodes concern respiratory system diseases.

There were no significant differences between the virological responders and non-responders in terms of age, number of previous schemes, number of ARV drugs associated with LPV/r, or PI use in the previous failed ARV therapy. The only factors that influenced immunological response were baseline age, baseline $CD4^+$ values, and number of ARV drugs associated with LPV/r. The univariate analyses showed that older children (median age=11.48 years) were less likely to develop an immunological response ($p<0.001$) than younger children (median age=5.2 years). Nine children receiving 3 ARV drugs plus LPV/r presented an immunological response (100%) in contrast to 10 of 20 (50%) children receiving 2 drugs ($p=0.01$). A lower number ($n<5$) of infectious episodes was observed in children after 12 months follow up using the LPV/r scheme [$p=0.006$; OR=0.16 (95% CI 0.03–0.76)].

Discussion

One of the most important characteristics of HIV-infected children treated using HAART is the lower virological response rates compared to adults. Further, achieving an undetectable viral load is a difficult goal in HIV-1-infected children undergoing antiretroviral therapy [11]. Our data show that HAART with LPV/r induces beneficial effects in terms of clinical, virological and immunological outcomes, even in children highly previously exposed to ARV therapy. Unexpectedly, the virological responses were not influenced by factors like number of previous schemes, number of ARV drugs associated with LPV/r or PI use in the previous failed ARV therapies. Excluding difficulties with adherence to the therapy, which were not assessed in this evaluation, we expected children who had taken more drugs, and those who had received PIs prior to LPV/r treatment, to have poor virological responses; however, our data do not confirm this supposition. Differently from our results, Resino et al found that children with virological failure or VL rebound had a higher baseline VL and lower $CD4^+$ T-lymphocyte count/mm^3, and had taken a greater number of drugs prior to LPV/r [11]. Another study showed that baseline plasma HIV-1 RNA levels affect the likelihood of achieving sustained viral suppression. Also, durable virological and immunological benefits were more likely to be achieved in children whose $CD4^+$ lymphocyte counts increased > 70 cells/mL by 20 weeks of therapy [12]. Although LPV/r has been considered suitable for 'salvage' therapy because of its high barrier to the development of resistance, most children studied (86%) were highly ARV-experienced, and had probably accumulated several PI-associated, resistance mutations, which may explain the high percentage of children (44.8%) with virological failure.

Importantly, we have observed that, in agreement with previous studies using HAART with different PIs [5,13-15], CD4+ T cells increased significantly in most children (65%), irrespective of the extent of virological suppression. Also, three factors influenced the immunological responses: the baseline age of the children, baseline $CD4^+$ values, and number of ARV drugs associated with LPV/r. The fact that older children (median age=11.48 years) were less likely to develop an immunological response than younger children (median age=5.2 years) probably reflects significant impairment of the immune system in the latest stages of HIV infection. Further, thymus function is critical for the regeneration and reconstitution of T-cell populations in hosts subjected to prolong, extreme T-cell depletion. We observed a significant increase in the percentage of children exhibiting an immunogical response when a more potent therapy was used. Nine children receiving three ARV drugs plus LPV/r showed immunological response (100%), in contrast to 10 of 20 (50%) children receiving two drugs plus LPV/r ($p=0.01$). A recent study has correlated immune system recovery in heavily pretreated, HIV-infected children, in response to salvage therapy with LPV/r, to a decrease in immune system activation and an increase in thymus function [16]. Further, restoration

of thymus function and a higher thymus output may play a critical role in sustaining peripheral $CD4^+$ cell increases despite the persistence of viral replication. In the present study, although the use of a more aggressive therapy (three *versus* two drugs plus LPV/r) is associated with a better immunological response, aspects such as toxicity, adverse events and the quality of life should be considered [5,17].

The clinical impact of HAART with LPV/r in the population studied could also be seen in terms of the reduction in the number of infectious events. After 12 months observation, the children showed a lower number ($n<5$) of infectious episodes [$p=0.006$; OR=0.16 (95% CI 0.03–0.76)]. Shifts in co-receptor usage (from SI/X4 to NSI/R5) after LPV/r salvage therapy have been related to clinical efficacy [18].

The most frequent infections observed in this group were URI/ common cold (31%), otitis (17.3%) and pneumonia (11%). Although some of these afflictions are considered "common childhood infections", they can have a serious impact on immunodeficient children infected with HIV. The small number of opportunistic infections found during the study period (one case of CMV, one case of cryptococcosis and 3 cases of herpes zoster) did not allow separate analysis.

In summary, the efficacy of highly active antiretroviral therapy is strictly dependent on its ability to control HIV-1 replication. Issues regarding potency of the employed drugs, adherence to therapy and development of drug resistance are the major determinants of treatment failures. In this study, HAART including LPV/r provided beneficial effects in terms of clinical, immunological and virological outcomes even in highly ARV experienced children.

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