Genotype-guided antiretroviral regimens in children with multidrug-resistant HIV-1 infection

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BACKGROUND: Genotyping tests were developed to attenuate the impact of viral resistance. Information about the efficacy in genotype base antiretroviral therapy in children is rare and even more in low- and middle-income countries.

METHODS: Sixteen children with antiretroviral therapy (ART) failure and triple-class drug-resistant viruses were included in this study. Protease and retrotranscriptase genotypes were available for all patients. Switch of ART regimen was guided by genotyping data. The primary end point was virological suppression (<50 copies/ml) and immunological improvement after 48 wk of treatment with the new ART regimen.

RESULTS: The median age of the patients was 14.5 y (interquartile range (IQR) 11–16.5). Median HIV-1 RNA viral load was 4.2 log₁₀ (IQR: 3.4–4.8). The primary end point was found in 11 children (69%), and 13 children (81%) had an HIV-1 RNA viral load <200 copies/ml. Median (IQR) for the baseline CD4⁺ cell count was 382 cells/μl (281–686 cells/μl), whereas after 48 wk of treatment with the new ART regimen, it was 640 cells/μl (361–936 cells/μl) ($P < 0.001$).

CONCLUSION: Darunavir/r, raltegravir, and etravirine were well tolerated in the present pediatric population. These drugs provide good options for children exposed to extensive ART. Regimens guided by genotyping data were effective for children who had ART failure and multidrug-resistant HIV-1 infection.

Infants and children who were infected perinatally are now surviving to adulthood with lifelong HIV infection and long-term exposure to combined antiretroviral therapy (ART) (1). Data from the United Kingdom Collaborative HIV Pediatric Study cohort demonstrated that over one-third of children had experienced virological treatment failure and were on a second or subsequent line of therapy at the time of their transition to adult care (2). In the pediatric setting, virological treatment failure occurs most frequently because of poor adherence to ART, and whenever viral replication is inefficiently controlled, virological treatment failure occurs more quickly, allowing the selection of HIV-1 quasiespecies resistant to antiretroviral (ARV) drugs (3).

Many innate and external factors have been associated with HIV drug resistance mutations in children, such as being infected perinatally, and extremely high viral load during the first steps of the infection, which can take longer to suppress than that in older children or adults, even when they are on fully active ART regimens (4).

Interpretation of resistance tests in this context can be complex, particularly in the presence of expanding options for ARV drugs, and treatment decisions should be made with the support of pharmacists and clinicians who have expertise in the field (5).

Currently, more than 20 different ARV drugs with six different classes of action have been approved for use in children or adolescents with HIV infection worldwide; most of them are used in Mexico (6).

A good level of adherence is particularly difficult during adolescence, especially with a complex regimen if a once-daily low pill burden option is not available because of prior acquisition of resistance mutations (7).

Ritonavir-boosted darunavir (DRV/r) and raltegravir (RAL) represent potential choices for salvage ART in children and adolescents who have failed to respond to protease inhibitors (PIs) with high-grade PI resistance. Studies of children and adolescents with experience of ART have shown safety and tolerability of DRV/r in these younger populations (8,9). DRV/r has been widely recommended as a salvage therapy for children older than 3 y and adolescents with high levels of treatment experience and ART resistance (9).

The aim of the study was to assess the virological and immunological response to an individualized ARV regimen guided by genotyping data in HIV-1-infected children and adolescents with ART experience and resistance. Secondary metabolic effects of the regimen are described.

RESULTS

Sixteen patients were included. Their median age was 14.5 y (interquartile range (IQR): 11.2–16.7), and 10 were boys. Class B of the CDC classification system of AIDS was found in seven patients (44%), class C in 6 (37%), and class A in 3

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Received 12 October 2015; accepted 28 December 2015; advance online publication 13 April 2016. doi:10.1038/pr.2016.53

Table 1. Baseline characteristics (*N* = 16)

Characteristics	ART regimen			All patients (<i>n</i> = 16)
	DRV/r + OBR (<i>n</i> = 13)	TPV/r + OBR (<i>n</i> = 2)	Without PI (RAL, ETV, TDF) (<i>n</i> = 1)	
	Median (IQR)			
Duration of virological failure, years	7 (4–10)	5.5 (3–8)	10	7.5 (3.2–10)
Baseline HIV-1 RNA, log ₁₀	4.08 (2.43–4.8)	4.98 (3.55–5.27)	5.22	4.2 (3.47–4.88)
CD4 ⁺ cells count, cell/μl	350 (298–666)	663 (631–694)	276	382 (281–687)
CDC class, <i>n</i> (%)				
A	3 (24%)	0	0	3 (18.7%)
B	5 (38%)	2 (100%)	0	7 (43.7%)
C	5 (38%)	0	1 (100%)	6 (37.5%)

ART, antiretroviral therapy; DRV/r, darunavir; IQR, interquartile range; OBR, optimized background regimen; PI, protease inhibitor; RAL, raltegravir.

Table 2. History of antiretroviral treatment

	ART regimen			All patients (<i>n</i> = 16)
	DRV/r + OBR (<i>n</i> = 13)	TPV/r + OBR (<i>n</i> = 2)	Without PI (RAL, ETV, TDF) (<i>n</i> = 1)	
Median of previous ARV regimens (IQR)	5 (4–6)	4 (2–6)	7	5 (4–6)
Previous ARV treatment				
≥1 PI, <i>n</i> (%)	13 (100%)	2 (100%)	1 (100%)	16 (100%)
≥1 NNRTI, <i>n</i> (%)	10 (78%)	1 (50%)	1 (100%)	12 (75%)
≥2 NRTIs, <i>n</i> (%)	13 (100%)	2 (100%)	1 (100%)	16 (100%)
Baseline resistance				
Primary IAS—USA mutations ¹⁰				
PI RAMs	4 (1–5)	5 (3–6)	6	4 (1–6)
NRTI RAMs	4 (0–6)	3 (2–4)	4	4 (0–6)
NNRTI RAMs	1 (0–4)	2 (0–4)	2	1 (0–4)

ART, antiretroviral therapy; ARV, antiretroviral; DRV/r, darunavir; IAS-USA, International antiviral society—USA; IQR, interquartile range; NNRTI, non-nucleoside reverse-transcriptase inhibitors; NRTI, nucleoside reverse-transcriptase inhibitors; OBR, optimized background regimen; PI, protease inhibitor; RAL, raltegravir; RAMs, resistance-associated mutations.

(19%), and the median number of previous ART regimens was 5 (IQR: 4–6) (Tables 1 and 2). None of the patients included had opportunistic or other infections during the study.

When we analyzed the optimized background regimen according to the Stanford HIV database (HIVdb), we did not find any difference between the genotype sensitivity score (GSS) of optimized background regimen in those patients with virological treatment failure and those with HIV-1 RNA <50 copies/ml.

The median calculated GSS of the current regimen was 2.12 (IQR: 1.75–2.5). Twelve (75%) patients had a GSS ≥2 taking in to account all components included in prescribed regimen, or only two patients (13%) if we measure the GSS excluding a completely active component (RAL or DRV). There was no association between GSS and a greater possibility of treatment failure (*P* = 0.87; Table 3).

The median of baseline HIV-1 RNA was 4.20 log₁₀ (IQR: 3.47–4.88 log₁₀). After 48 wk of treatment, 69% had <50 copies/

ml, median 1.33 log₁₀ (IQR: 1.30–2.26 log₁₀), and 81% of the patients (*n* = 13) had an HIV-1 RNA <200 copies/ml; *P* < 0.001 compared with baseline.

For five patients with virological treatment failure, one had virological rebound, and four never achieved an HIV-1 RNA load of <50 copies/ml (1.69 log₁₀); two of these four had <200 copies/ml.

The median (IQR) for the baseline CD4⁺ cell count was 382 cells/μl (281–686 cells/μl), whereas after 48 wk of treatment, the value was 640 cells/μl (361–936 cells/μl). This increase was statistically significant (*P* < 0.001).

Fasting lipid profiles (total cholesterol and triglycerides) were not significantly different from basal levels after 48 wk of treatment. Hypercholesterolemia and hypertriglyceridemia were found in 12 and 20% of patients at baseline and in 25 and 50% after 48 wk of treatment (*P* = 0.50, 0.18), respectively.

There was no significant increase in creatinine or glucose compared with baseline values (Table 4).

Table 3. ART characteristics, optimized background regimen and outcomes

	ART regimen			All patients (n = 16)
	DRV/r + OBR (n = 13)	TPV/r + OBR (n = 2)	Without PI (RAL, ETV, TDF) (n = 1)	
GSS ARV therapy, n (%) ^a				
1 < 2	4 (30%)	0	0	4 (25%)
≥ 2	9 (70%)	2 (100%)	1 (100%)	12 (75%)
OBR GSS (RAL or DRV completely active), n (%)				
0 < 1	4 (31%)	—	—	5 (31%)
1 < 2	7 (54%)	—	—	9 (56%)
≥ 2	2 (15%)	2 (100%)	1 (100%)	2 (13%)
GSS ARV therapy, median (IQR)	2 (1.75–2.5)	2.38 (2.25–2.38)	1.75	2.12 (1.75–2.5)
OBR GSS (RAL or DRV completely active), median (IQR)	1 (0.75–1.50)	1.38 (1.25–1.50)	0.75	1.125 (0.75–1.5)
HIV-1 RNA viral load (<50 copies/ml) at week 48, n (%)	8 (61.5%)	2 (100%)	1 (100%)	11 (68.7%)
HIV-1 RNA viral load (<200 copies/ml) at week 48, n (%)	10 (77%)	2 (100%)	1 (100%)	13 (81.2%)
Three most used ARV in OBR, n (%)				
TDF	8 (62%)	1 (50%)	1 (100%)	10 (63%)
RAL	10 (77%)	2 (100%)	1 (100%)	13 (81%)
ETV	2 (15%)	0	1 (100%)	3 (19%)
Patients with virological failure, n (%)	5 (38%)	0	0	5 (31%)

ART, antiretroviral therapy; ARV, antiretroviral; DRV/r, darunavir; GSS, genotype sensitivity score; IQR, interquartile range; OBR, optimized background regimen; PI, protease inhibitor; RAL, raltegravir.

^aGenotypic score according to Stanford HIVdb.

Median weight-for-age and height-for-age (z-scores) were stable over the period of the study; all patients obtained or maintained WHO clinical treatment stage I ([Table 4](#)).

The most common drug resistance mutations present for nucleoside reverse-transcriptase inhibitors were M184V (81%), M41L (56%), T215Y/I/F (50%), K219Q/E (37%), and K70R (31%), for non-nucleoside reverse-transcriptase inhibitors were K103N (31%), Y188L (25%), and G190S/A (25%), and for PIs were L54V (100%), V82A/S (75%), M46I/L (69%), and L90M (44%) ([Table 5](#)).

We were not able to evaluate adverse events fully because of the retrospective study design. However, the regimen was not suspended because of side effects in any of the children.

DISCUSSION

We showed the feasibility of achieving a virological and immunological response with an antiretroviral regimen guided by genotype in this cohort of Mexican children and adolescents infected with HIV perinatally, even after the selection of triple-class drug resistance mutations. All patients who achieved viral suppression showed improved or stable CD4⁺ cell counts, even after long-standing treatment failure. At the end of this study, all of the patients in this report were classified as having a WHO class 1 HIV infection, and they did not present opportunistic infections during the study; however, the prescription of an effective combination therapy for HIV-infected children harboring an MDR virus is one of the greatest challenges in the clinical management of children with HIV infection (11,12). In this study, the basal sample had a susceptibility to DRV,

Tipranavir (TPV), Etravirine (ETV), and Tenofovir (TDF) of 87, 37, 87, and 50%, respectively, according to the Stanford HIVdb; in addition, RAL was considered to be fully active.

Routine clinical use of DRV/r and/or RAL in children in hospitals with or without economic resources is limited: a cohort in Botswana of four TC-MDR pediatric patients on DRV/r and/or RAL-based regimens all achieved viral suppression showed improved/stable CD4⁺ cells counts and obtained or maintained WHO clinical treatment stage I, even after long-standing virological/immunological failure (11). We are not aware of previously published reports from Latin America or Mexico. Our data are similar to those from a French cohort where 11/12 adolescents with extensive experience of ARV treatment achieved viral suppression (<400 copies/ml) with a regimen of DRV/r plus RAL plus ETV (12). Despite ARV resistance, the susceptibility to some drugs, such as new PIs (TPV and DRV) and non-nucleoside reverse-transcriptase inhibitors (ETV and rilpivirine), was maintained in a cohort of Spanish children and adolescents with perinatally acquired HIV infections and TC-MDR (13).

Some studies show the efficacy of some ARV drugs used separately in children, such as the phase 2 PIANO study that shows the efficacy of ETV treatment in HIV-1-infected children and adolescents who previously used EFV or NVP; 56% of patients (68% children; 48% adolescents) achieved virological response (14). In another trial, RAL was evaluated as part of a salvage treatment in a multicenter retrospective study of 19 MDR children and adolescents. After receiving RAL-based therapy, 4/19 (21%) patients achieved an HIV-1 RNA load of

Table 4. End points after 48 wk of treatment

Outcomes	Median (IQR)		P
	Baseline	Week 48	
CD4 ⁺ cells count, (cells/ μ l)	382 (281–687)	640 (361–936)	0.001
HIV-1 RNA viral load ($\log_{10}$)	4.20 (3.47–4.88)	1.33 (1.30–2.26)	<0.0001
Weight, Z score	–1.66 (–2.24, –0.80)	–1.46 (–2.05, –0.81)	0.932
Height, Z score	–2.05 (–2.87, –1.13)	–1.75 (–2.72, –1.41)	0.187
BMI, Z score	–0.36 (–1.56, 0.20)	–0.3 (–2.04, 0.07)	0.650
Cholesterol (mg/dl)	145 (137–171)	165 (133–204)	0.196
Triglycerides (mg/dl)	118 (86–143)	149 (79–191)	0.112
Creatinine (mg/dl)	0.5 (0.42–0.67)	0.48 (0.39–0.57)	0.141
Glucose (mg/dl)	83 (75–91)	85.8 (79–90.3)	0.382

IQR, interquartile range.

<400 copies, and 13/17 (68%) reached an HIV-1 RNA load of <50 copies; CD4⁺ cell count recovery was observed in 17/19 (89%) patients. No deaths, AIDS-defining illnesses, or symptoms of severe intolerance were recorded in this multicenter cohort (15).

In this study, a mild increase in lipids was found, but this was not statistically significant, maybe because of the previous use of other PIs having a worse effect on lipids. The increase in lipids was higher in teens than in children (58 vs. 12%), and it was probably associated with longer exposure to the ART, hormonal changes, and more flexible diets, as described in other reports (16).

Children infected perinatally are usually exposed to many ART regimens, and once in adolescence and young adulthood, they have typically accumulated drug-resistant viruses that can be significantly difficult to control (17). Infants have a higher risk of developing resistance compared with adolescents and adults because younger children have higher viral loads, and during treatment, their family members cannot provide accurate dosing, because of the limitations in formulations of antiretroviral drugs, which can lead to subtherapeutic drug concentrations (17).

There are a considerable number of children and adolescents who acquired their HIV-infection in the pre-HAART (highly active antiretroviral therapy) era. Fifty percent of patients in the present cohort (eight patients) had received a large number of antiretroviral regimens, with a median of five regimens in the entire cohort; consequently, drug resistance mutations complicate selection of new ART regimens (18).

There are now six therapeutic classes of medications available, although not all are licensed for use in children, and some of them are not available in Mexico (e.g., dolutegravir, cobicistat). Most ARV (developed in tablet form) are impractical for children aged less than 5 y, and other options, such as special liquid formulations, small pills, or chewable formulations, are not all available yet, and if they do exist, they are not available in Mexico. Although older children can take tablets, adult doses are often too large (19). The main reason for multiple

Table 5. Major drug-associated mutations

Mutation	n (%)
Associated with NRTIs	
L184V	13 (81%)
M41L	9 (56%)
T215Y/I/F	8 (50%)
K219Q/E	6 (37%)
D67N	5 (31%)
K70R	5 (31%)
T69N	3 (19%)
L74V	1 (6%)
V75M	1 (6%)
Associated with NNRTIs	
K103N/S	5 (31%)
G190A/C/S/V	4 (25%)
Y188C/L	4 (25%)
K101E/P	2 (12%)
Y181C	1 (6%)
Associated with PIs	
L54L/V	16 (100%)
V82A/S	12 (75%)
M46I/L	11 (69%)
L90M	7 (44%)
I84V	6 (37%)
T74A/P/S	3 (19%)
L76V	3 (19%)
G48V	2 (12%)
L50V	2 (12%)
Q58E	2 (12%)
V32I	1 (6%)

NNRTI, non-nucleoside reverse-transcriptase inhibitors; NRTI, nucleoside reverse-transcriptase inhibitors; PI, protease inhibitor.

regimens before genotyping for salvage therapy in this population was the lack of a pediatric indication for new ARV drugs recently accepted for pediatric use.

Although our experience reported here involves a small number of patients, sustained virological suppression has been achieved in patients who previously experienced persistent viremia with complete adherence to current ART; immunological indices improved or they were stable, and no intolerance or toxicity was noted in any patient, including when high levels of resistance to PIs were present. No deaths or AIDS-defining clinical conditions were observed in our cohort during the salvage regimen.

Genotypic resistance testing following virological treatment failure in adults has been shown to lead to modest virological benefits (20,21). PENTA 8 was a randomized trial of genotypic resistance testing vs. clinician-guided switch in children with failing virological treatment, and this study showed no

virological or immunological benefit at 96 wk (22); however, this finding should be interpreted with caution, because second-generation PIs and TDF were not available at the time of the study. In the present series, a favorable response observed in 13 of 16 patients who received combination therapy with the salvage drugs could be attributed to an individualized analysis of genotype by an expert committee and recommendation of the individualized ARV regimen for each patient.

The present study has some limitations; these include the small sample size, retrospective method, short follow-up time, and possible selection bias because of the type of the hospitals, which are centers of reference.

This population is increasing within the time, and the analysis continues.

In conclusion, there are specific challenges in treating HIV-infected children, including uncertainty about the best time to start treatment, the need for more pediatric formulations, the lack of pharmacokinetic studies for new drugs in children, and incomplete dosing guidelines for children. The genotypic resistance testing is a valuable tool to conform an effective antiretroviral regimen in highly experimented children.

METHODS

Design

A retrospective multicenter study of a cohort of HIV-1-infected children with ART experience was conducted. The first end point analyzed was an HIV-1 RNA viral load of <50 copies/ml after the patients had completed 48 wk of treatment. The secondary end point was an HIV-1 RNA viral load of <200 copies/ml and any increase in the CD4⁺ cell count.

The Institutional Review Board and Ethics Local Committee in the Hospital de Pediatría CMN SXXI reviewed and approved this study, with reference number: R-2012-3603-81.

Patients

Children aged <18 y with an HIV-1 infection acquired perinatally and ART failure were included. Patients were previously treated with at least three classes of ARV drugs including nucleoside analog reverse-transcriptase inhibitors, non-nucleoside reverse-transcriptase inhibitors, and PIs, with documented triple-class drug resistance mutations. Due to the retrospective nature of the study, written informed consent was not necessary.

An individualized ART was designed for each patient. The regimen included three to four ARV agents, with two or more fully active drugs according to HIV-1 resistance testing and previous ARV drug experience.

Measurements

Clinical history regarding ARV regimens, CD4⁺ cell counts, HIV-1 RNA viral load, and serum laboratory parameters at the beginning of the therapy and at 24 and 48 wk later were recorded. After genotyping, and considering previous use of ARV regimens, an expert committee evaluated each case to determine the best option for a salvage regimen.

Mutations were assessed from plasma HIV-1 *pol* sequences using the Stanford HIVdb. The presence of resistance was defined according to Stanford HIVdb (SS) ranges as follows: 0–9, susceptible; 10–14, potential low-level resistance; 15–29, low-level resistance; 30–59, intermediate resistance; and ≥60, high-level resistance.

The GSS is based on the total number of drugs in an ARV regimen to which the HIV isolate has genotypic sensitivity, as deduced from gene sequence and mutation analyses. This was calculated based on the drug resistance scores extracted from the Stanford HIVdb. Each ARV drug was assigned a score according to the five-level Stanford HIVdb interpretation: 1.00 (susceptible), 0.75 (potential low-level resistance), 0.50 (low-level resistance), 0.25 (intermediate resistance), and 0.00

(high-level resistance). The GSS is defined as the sum of all scores for all the drugs in the regimen. The arithmetic sum of the individual score for the specific drugs provided the total GSS for that treatment. We classified the total GSS score into the following categories: <1, 1 to 2, and ≥2. The 0 to 1 group contains viral sequences almost entirely resistant to the drugs in their regimen, and the ≥2 group contains viral sequences susceptible to two or more drugs given in their ARV regimen.

The effectiveness of ART was evaluated using the percentages of undetectable HIV-1 RNA viral load after 48 wk of treatment. Changes in CD4⁺ cell counts were also evaluated.

Metabolic end points were changes in fasting lipid levels (total cholesterol and triglycerides), in glucose, and in creatinine from baseline and at 48 wk. Including weight, height, and BMI Z scores before and at final analysis.

Statistical Analyses

Baseline characteristics are summarized using medians and IQR for continuous variables and proportions for categorical variables.

The differences in the CD4⁺ cell count, HIV-1 RNA viral load, and lipids, at baseline and after 48 wk of treatment were calculated. Because the data followed an irregular distribution, they were compared using a Wilcoxon signed-rank test. A Mann–Whitney *U*-test was used to evaluate differences between ARV GSS scores, and viral and immunological end points.

All analyses were conducted using SPSS software (IBM SPSS for Windows, version 19.0, 2010 release, IBM, Armonk, NY).

STATEMENT OF FINANCIAL SUPPORT

No financial assistance was received to support this study.

Disclosure: There are no disclosures.

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