Response to a protease-inhibitor (ritonavir)-containing combination antiretroviral regimen in HIV-infected children

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INTRODUCTION: The number of antiretroviral agents available for children who are failing existing therapy is limited. Data are lacking on the use of various combination regimens and the resulting viral load dynamics in such children.
METHODS: Between March 1998 and March 2000, HIV-infected children younger than 18 years of age were studied in an open trial. The study regimen included ritonavir, with at least two drugs to which the virus was known or presumed to be sensitive. Subjects were ritonavir-naive and were included if they had high viral loads while receiving antiretroviral therapy. Patients had clinical assessments, CD4 counts and viral load monitoring.
RESULTS: Fifteen antiretroviral-experienced HIV-infected children were enrolled. Approximately 87% (13 of 15) had perinatally-acquired HIV; median age was 7.9 years (range 1.6 to 14.8). At enrolment, the median CD4 count was 557 cells/mm^3 (range 57 to 1702) and the median viral load was 72,600 copies/mL (range 3626 to 796,440). The majority of children (73.3%) had increases in CD4 counts within 12 weeks. During this period, the median increase in CD4 counts over baseline was 30.0%. Approximately 73% (eight of 11) of subjects with initial improvements in CD4 counts had sustained increases at 32 to 48 weeks. Over the first 12 weeks, 60% (nine of 15) had greater than 0.5 $\log_{10}$ decreases in viral load. The improvement was sustained in 88.9% (eight of nine) of these patients at 32 to 48 weeks. Three patients discontinued therapy due to taste aversion.
CONCLUSIONS: Among pediatric patients with high viral loads while on existing therapy, the ritonavir-containing regimen was generally well tolerated. In a significant proportion of patients, modification of therapy was associated with sustained improvements in viral loads and CD4 counts over 32 to 48 weeks.

Key Words: *Antiretroviral therapy; HIV infection; HIV viral load; Pediatrics; Protease inhibitor*

La réponse à une association d'antirétroviraux contenant un inhibiteur de la protéase (ritonavir) chez des enfants infectés au VIH

INTRODUCTION : Le nombre d'antirétroviraux à la disposition des enfants qui ne réagissent pas aux traitements existants est limité. Il n'existe pas de données sur l'utilisation des diverses posologies d'associations et sur la dynamique de la charge virale résultante chez ces enfants.
MÉTHODOLOGIE : Entre mars 1998 et mars 2000, des enfants infectés au VIH de moins de 18 ans ont fait l'objet d'une étude en essai ouvert. La posologie à l'étude incluait le ritonavir, ainsi qu'au moins deux médicaments auxquels on savait ou présumait le virus sensible. Les sujets n'avaient jamais pris de ritonavir et étaient enrôlés s'ils présentaient une charge virale élevée malgré un traitement aux antirétroviraux. Les patients bénéficiaient d'évaluations cliniques, de numérations des lymphocytes T-CD4 et d'une surveillance de leur charge virale.
RÉSULTATS : Quinze enfants infectés au VIH ayant déjà pris des antirétroviraux ont participé à l'étude. Environ 87 % (13 sur 15) étaient atteints d'un VIH acquis pendant la période périnatale, leur âge médian était de 7,9 ans (entre 1,6 an et 14,8 ans). À leur enrôlement dans l'étude, la numération de lymphocytes T-CD4 était de 557 cellules/mm3 (entre 57 et 1 702) et la charge virale moyenne était de 72 600 copies/mL (de 3 626 à 796 440). La majorité des enfants (73,3 %) présentaient des élévations de leur numération de lymphocytes T-CD4 au bout de 12 semaines. Pendant cette période, l'augmentation moyenne des numérations de lymphocytes T-CD4 au-dessus de la base de référence était de 30,0 %. Environ 73 % (huit sur 11) des sujets affichant une amélioration initiale des numérations de lymphocytes T-CD4 présentaient des augmentations soutenues entre 32 et 48 semaines. Au cours des 12 premières semaines, 60 % (neuf sur 15) affichaient des diminutions de la charge virale supérieure à 0,5 $\log_{10}$. L'amélioration était maintenue chez 88,9 % (huit sur neuf) de ces patients entre 32 et 48 semaines. Trois patients ont interrompu leur traitement en raison du mauvais goût du médicament.
CONCLUSIONS : Parmi les patients pédiatriques présentant une charge virale élevée malgré un traitement en place, la posologie contenant du ritonavir était généralement bien tolérée. Chez une proportion significative de patients, la modification du traitement s'associait à des diminutions soutenues de la charge virale et à une amélioration soutenue des numérations de lymphocytes T-CD4 entre 32 et 48 semaines.

Antiretroviral regimens for use among individuals who are failing antiretroviral therapy have largely been evaluated in adults. Protease-inhibitor-containing regimens have had profound beneficial effects in suppressing viral replication (1-4). Modification of therapy usually entails changing at least two agents, sometimes including a protease inhibitor (5-6). While such regimens have also been shown to be beneficial in children (7-11), several questions remain unanswered from a pedi-

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TABLE 1
Baseline characteristics of subjects at enrolment

Variable	Value
Median years of age (range)	7.9 (1.6 to 14.8)
Sex (Male:Female ratio)	1:1.5
Perinatally-acquired HIV	13 of 15 (86.7%)
Median duration (years) of previous antiretroviral therapy (range)	2.3 (1.2 to 5.8)
Median CD4 count (cells/mm^3) (range)	557 (57 to 1702)
Median viral load (copies/mL) (range)	72,600 (3626 to 796,440)
Number with previous opportunistic infections or conditions (%)	11 of 15 (73.3)
Number previously treated with protease inhibitor (%)	9 of 15 (60.0)
Disease stage (%)	
N1	2 (13.3)
N3	1 (6.7)
A2	2 (13.3)
B1	1 (6.7)
B2	2 (13.3)
C3	7 (46.7)
Number of baseline antiretroviral agents (%)	
Two nucleoside reverse transcriptase inhibitors	6 of 15 (40.0)
Two nucleoside reverse transcriptase inhibitors and one protease inhibitor	7 of 15 (46.7)
Other	2 of 15 (13.3)

atric perspective. In this context, it is important to document the effectiveness of various regimens in lowering viral load in infected children as well as to explore the reasons why, in some children, the success rates are suboptimal with protease-inhibitor-containing antiretroviral therapy (11).

Ritonavir is one of the most widely used protease inhibitors in Canada. The present study evaluated ritonavir-containing combination regimens that were employed in a setting where patients were failing existing therapy by virtue of having persistently high viral loads. The objectives of the study were: first, to determine if a treatment strategy employing triple or quadruple combination antiretroviral therapy containing ritonavir would result in significant reductions in HIV-1 ribonucleic acid (RNA) levels in a population of previously treated HIV-infected children; second, to compare the characteristics of patients whose viral loads responded to modification of therapy with those who failed to respond; and third, to examine the effects of the above regimen on changes in CD4.

METHODS

An open nonrandomized intervention trial that involved three Canadian tertiary centres affiliated with the Canadian Pediatric AIDS Research Group (CPARG) was conducted. The study was conducted between March 1998 and March 2000 and subjects were followed for a maximum duration of 48 weeks.

HIV-infected children were eligible if they were younger than 18 years of age and were experienced with antiretroviral therapy, but were ritonavir-naive. Informed consent was obtained. They were eligible for enrolment if their physicians assessed them as failing their existing antiretroviral therapy by virtue of having persistently elevated HIV-1 RNA levels.

TABLE 2
CD4 count and viral load responses within 12 weeks of treatment modifications

Variables	Values
Median per cent change in CD4 count (ranges)	
All subjects	23.7 (–26.3 to 346)
Responders	30.0 (6 to 346)
Nonresponders	–13.8 (–7.7 to –26.3)
Number (%) with change in CD4 count of:	
≥20% above baseline	8 of 15 (53.3)
≥20% below baseline	1 of 15 (6.7)
Log_{10} reduction in viral load (%)	
≥0.5	9 of 15 (60.0)
≥1.0	9 of 15 (60.0)
≥1.5	8 of 15 (53.3)

All subjects were seen initially and were then followed at an interval of approximately monthly for six months, and then every two months for six months at which time they had clinical, virological and immunological assessments. HIV-1 RNA levels and CD4 counts were performed. HIV-1 RNA levels were performed in regional references laboratories using the Chiron (Chiron, USA) B DNA assay (lower limit of detection less than 50 copies/mL). The planned antiretroviral study regimen consisted of two new agents to which the virus was presumed to be sensitive, one of which was ritonavir.

Statistical analyses

Using the binomial test of proportions, the sample size was based on an estimated success rate of 80% with lower and upper bounds of ±20% based on a 95% confidence interval. This resulted in a target sample size of 15 subjects. Wherever appropriate, means were compared with Student's *t* test and medians with a nonparametric procedure (Kruskal-Wallis). Proportions were compared using χ^2 or Fisher's exact test as appropriate.

RESULTS

The baseline characteristics of the patients enrolled are shown in Table 1. Among 15 children, the majority of whom had perinatally-acquired HIV, the median age was 7.9 years (range 1.6 to 14.8). The median CD4 count was 557 cells/mm^3 (range 57 to 1702), while the median viral load at enrolment was 72,600 copies/mL (range 3626 to 796,440). Patients were on antiretroviral therapy for a median of 2.3 years (range 1.2 to 5.8). The majority (73.3%) had experienced an opportunistic condition before enrolment. The classes of the antiretroviral agents that the patients were receiving at enrolment are shown in Table 1. In this regard, the majority of patients were either receiving two nucleoside reverse transcriptase inhibitors (NRTIs) plus a protease inhibitor other than ritonavir (46.7%) or two NRTIs (40%). Zidovudine and lamivudine were the most frequently used NRTIs. Overall, 60% had received a protease inhibitor other than ritonavir before enrolment.

Eleven patients followed the study regimen. Four additional patients were enrolled, but the only change in therapy was the introduction of ritonavir to replace another protease

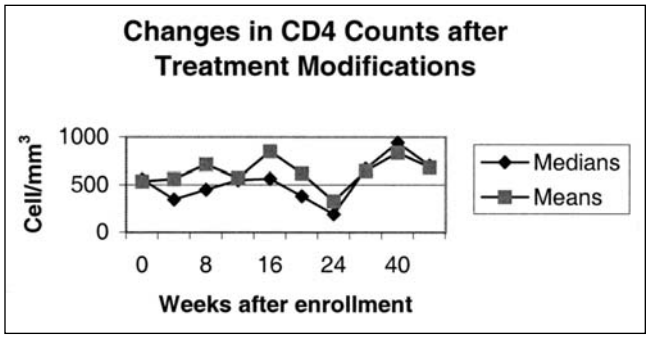

Figure 1) *Changes in CD4 counts after treatment modifications*

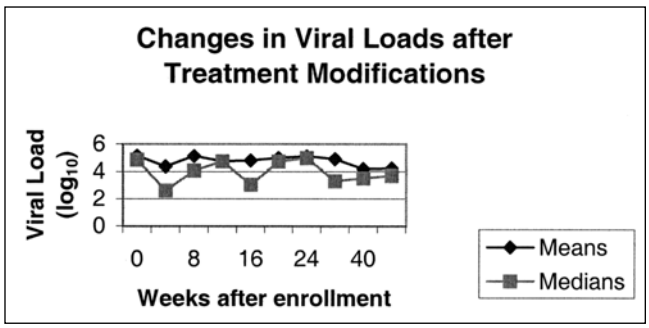

Figure 2) *Changes in viral loads after treatment modifications*

inhibitor. The results were analyzed on all 15 patients on an intent-to-treat basis. An initial improvement in CD4 counts was observed in 73.3% of patients. As shown in Table 2, 53.3% of subjects have an increase of greater than 20% in the CD4 counts over the first 12 weeks, while 6.7% experienced a corresponding decrease in CD4 counts. For all patients, the median percentage change in CD4 was 23.7% (range –26.3% to 346%). For those patients who had an initial response over the first 12 weeks, the median percentage change was 30.0% (range 6% to 346%). The corresponding changes among patients who did not show an initial CD4 response were median –13.8% (range –26.3% to –7.7%).

Two-thirds of the patients (10 of 15) experienced initial reductions in viral load of at least a 0.5 $\log_{10}$. In nine patients, this reduction occurred within the first 12 weeks, while one patient responded at 16 weeks. The proportions of patients who experienced specific degrees of reduction in viral load over the first 12 weeks are shown in Table 2.

We examined the extent to which changes in CD4 counts and viral loads were sustained. These are shown in Figures 1 and 2, respectively. Among subjects who showed initial improvements in CD4 counts, eight of 11 (72.7%) had sustained improvements through to 32 to 48 weeks. At weeks 32 to 48, the overall improvement in CD4 counts was 25% above the median baseline of 557 cells/mm^3.

For the group as a whole, there was a reduction in median viral load of 1.2 to 1.6 logs between baseline and weeks 32 to 48. Eighty per cent of subjects who had initial reductions in viral loads had sustained improvements through to 32 to 48 weeks.

We examined the baseline characteristics of patients who experienced a significant initial reduction in viral load (0.5 log or greater) with those who did not have a significant decrease.

TABLE 3
Potential predictors of the likelihood of a virological response to modification of therapy

Variables at enrolment	Responders* (n=10)	Nonresponders (n=5)	P
Age in years (median [range])	7.2 (1.6-14.8)	8.8 (5.2-12.8)	0.33
Duration or prior antiretroviral therapy in years (median [range])	2.2 (1.6-5.8)	3.8 (1.2-5.2)	0.27
CD4 count (cells/mm^3) (median [range])	614 (180-1702)	120 (57-558)	0.014
Viral load (copies/mL) (median [range])	69,405 (3626-796,440)	94,774 (16,859-500,100)	0.71
Previous protease inhibitor use	6 of 10	3 of 5	1

**Nine patients had a significant response within the first 12 weeks, while one patient responded at 16 weeks*

These characteristics are shown in Table 3. There was no significant difference in median ages between responders and nonresponders (median 7.2 years; range 1.6 to 14.8 versus 8.8 years; range 5.2 to 12.8, respectively, P=0.33). While the median baseline viral load was higher in the nonresponders than in the responders, the difference was not significant (94,774 versus 69,405 copies/mL, respectively, P=0.71). However, responders had higher median baseline CD4 counts compared with nonresponders 614 cells/mm^3 (range 180 to 1702) versus 120 (range 57 to 558, P=0.014). The duration of previous antiretroviral therapy was not significantly different between responders and nonresponders (medians 2.2 versus 3.8 years, P=0.27).

The differences between responders and nonresponders were re-examined by excluding the one patient who received ritonavir for less than three days, which was not long enough to assess effectiveness. The results were similar to those in Table 3 (P=0.007 for differences in CD4).

In 11 of 15 patients, the change in treatment regimen at enrolment resulted in the addition of at least two new agents, one of which was the protease inhibitor, ritonavir. Of these 11 patients, seven had a significant reduction in HIV-1 RNA levels of at least 0.5 log over the first 12 weeks. Four patients had a switch in the choice of protease inhibitor therapy as the only change. These four patients represented deviations from the planned protocol. Of these four patients, one had a reduction in viral load of at least 0.5 log over the first 12 weeks, but with no increase in CD4 counts.

Twenty per cent of the patients experienced no adverse events that were temporally associated with the use of the ritonavir-containing combination regimen. Twelve patients (80%) had the following events documented while they were on the study regimen: grade 1 toxicity with peripheral neuropathy (two of 12); anemia at a grade 1 or 2 level (three of 12); deranged liver function tests at a grade 3 or 4 level (five of 12); elevated serum lipase at a grade 3 level (one of 12) and neutropenia at a grade 2 level (one of 12). However, five of these 12 patients had evidence indicating that the onset of these abnormalities occurred before the initiation of the riton-

avir-containing regimen. While the majority of subjects tolerated ritonavir, taste aversions occurred in three subjects.

DISCUSSION

Among HIV-infected children, it has been shown that changes in CD4 counts and HIV-1 RNA levels are independent predictors of disease progression and survival (12). In this study involving HIV-infected children who were failing existing antiretroviral therapy, we demonstrated that a significant proportion respond to a revision of the antiretroviral agents, including a protease inhibitor (ritonavir) as part of a combination regimen. We documented that changes in CD4 counts and viral loads occurred within the first 12 weeks after therapy was modified. The majority of patients who responded had greater than a 1.5 log decrease in viral load.

While the initial response to a modification in treatment is important, it is also worthwhile to examine whether the improvements seen are sustained. We were able to show that the majority of patients who experienced improvements in CD4 counts and viral loads had sustained responses for up to 32 to 48 weeks after modification of their treatments.

In a large randomized trial, Nachman et al (11) demonstrated the beneficial effects of a ritonavir-containing antiretroviral regimen. The authors noted that triple therapy containing ritonavir was just greater than 50% effective in maximally suppressing the viral load (11). Our findings are consistent with these results. In our study, close to two-thirds of patients had an initial response to a switch in treatment to a ritonavir-containing regimen. However, to provide some insight into the reasons for the lack of response in some patients, we examined several factors, including HIV-1 RNA levels and CD4 counts. Others have shown that, in pediatric cohorts, the virological responses do not necessarily correlate with baseline HIV-1 RNA levels (13). Likewise, we found no significant difference in baseline viral loads between patients who had reductions on HIV-1 RNA levels compared with those who did not have reductions in such levels. However, other investigators have observed that among patients receiving protease inhibitor-based salvage therapy, lower plasma HIV-1 RNA levels at the time of switching treatment were associated with improved outcomes (11,14,15).

Low baseline CD4 counts have been shown to be predictive of virological failure in response to protease inhibitor-containing salvage therapy (14). In our study, we demonstrated that patients with lower CD4 counts were less likely to respond to changes in treatment. This may help to define a group of children who should be given priority for antiretroviral resistance testing, particularly if they are failing existing therapy.

We assumed that the level or resistance to ritonavir would be low in a ritonavir-naive population. Thus, we felt that lack of responses would not be expected to be primarily due to drug resistance affecting ritonavir. However, it is likely that there was resistance to other components of the combination regimen. We have previously demonstrated that, among patients in the CPARG cohort, resistance to the nucleoside analog zidovudine was associated with lower CD4 counts and a longer duration of therapy (16).

While the majority of patients tolerated ritonavir, taste aversion was noted. Deranged liver function tests were among the most common features of the adverse events profiles documented. In the patients in whom the onset of ritonavir therapy was followed by adverse events, these events were consistent with the known side effects of this drug (4,17). However, it should be noted that in this study we did not conclusively show that these events were due to ritonavir as opposed to concomitant antiretroviral agents. The study is limited by sample size. This is a feature of many pediatric studies involving antiretroviral agents. In addition, it would have been ideal to have obtained serial testing of drug resistance on all subjects. In this context, we performed resistance testing on a subset of patients, but data were insufficient to allow for adequate quantitative analyses. The study was not designed to separate out the therapeutic effects of ritonavir from other antiretroviral agents, because the plan was to change more than one agent at the same time. In a minority of cases where there was a deviation from the study protocol that resulted in a change in the protease inhibitor as the only treatment modification, the therapeutic effect over the first 12 weeks was suboptimal (no significant viral load reduction and/or declining CD4 counts). This is consistent with what is expected given the known disadvantages of adding a single new agent to a failing regimen (6,18).

CONCLUSIONS

In summary, a ritonavir-containing combination antiretroviral regimen was beneficial for the majority of children who were failing existing therapy. Because benefit was not observed for some children, further studies need to be done to examine the characteristics of the children who fail to respond to salvage regimens and the role of novel combinations of antiretroviral agents, including protease inhibitors in these patients. Such efforts will require the cooperation of several centres, using similar protocols in a manner similar to many pediatric oncology studies.

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