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Oral Lactoferrin in HIV-1 Vertically Infected Children: an Observational Follow-up of Plasma Viral Load and Immune Parameters

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Lactoferrin (LF) is a mammalian iron-binding glycoprotein with antiviral effects. This preliminary study evaluated 6 months' LF (3 g/day, orally) treatment in 22 human immunodeficiency virus type 1 (HIV-1) vertically infected children. Plasma viral load and CD4+ cell counts were assessed every 3 months; before, during and after LF administration. No significant changes were observed during the pre-treatment period. By 6 months, mean (± SD) plasma viral load (log_{10}) declined from 4.54 (± 0.65) to 4.28 (± 0.60); median percentage CD4+ cell count increased from 21.5% to 24.5%. Two months after treatment discontinuation, mean plasma viral load did not differ significantly from baseline or month 6 levels, but the percentage CD4+ cell count remained significantly higher than the baseline value. LF plus antiretroviral (ARV) therapy was more effective at increasing CD4+ cell count than LF alone. None of the patients showed any new HIV-1-related symptoms at follow-up. LF might be a useful addition to ARV therapy, but further large-scale studies are required.

KEY WORDS: Lactoferrin; Antiretroviral drugs; Human immunodeficiency virus; Children

Introduction

Paediatric human immunodeficiency virus (HIV) infection has been transformed by antiretroviral (ARV) therapy into a chronic disease, although associated problems, such as side-effects of therapy, heavy pill burden and poor patient compliance, have raised many questions.

Recently, new compounds such as lactoferrin (LF), which do not have the disadvantages of synthetic drugs, have been evaluated to see whether they improve individual patient responses above those seen with ARV therapy.[1] LF is an iron-binding glycoprotein present in exocrine secretions and in mother's milk,[2,3] whose iron binding capacity might explain its bacteriostatic activity. The antibacterial action of LF is also achieved through an iron-independent bactericidal mechanism.[4,5] LF has shown anti-infective properties against fungal, viral and bacterial infections.[6] With regard to its antiviral properties, LF prevents

the penetration of envelope-containing viruses (cytomegalovirus, herpes simplex virus 1, adenovirus and HIV) into the target cell, while LF directly inhibits viral replication by binding to the virus in viruses without envelopes (e.g. rotavirus).[7–9] LF may also play an important role in the immune response by either acting directly on the immune system[10] or by indirectly acting on gut flora via a prebiotic effect.[11]

Lactoferrin has been shown *in vitro* to have an antiviral effect against HIV-1 infection,[12] but a possible protective effect *in vivo* has only been postulated once before.[13] We have performed a preliminary study in HIV-1 vertically infected children to investigate the effect of oral LF supplementation on plasma viral load and CD4+ cell counts by evaluating these parameters before, during and after LF supplementation.

Patients and methods

STUDY DESIGN

This preliminary study aimed to evaluate plasma viral load and CD4+ cell counts in HIV-1 vertically infected children before, during and after a 6-month period of oral LF supplementation. The study also evaluated the response of HIV-infected children to LF supplementation according to the ARV therapy that they were receiving at the time of enrolment. All HIV-infected children in this study remained on any existing ARV therapy throughout the study period.

PATIENT POPULATION

Children were recruited to the study by the Paediatric Department of San Paolo Hospital (Milan, Italy) and the Paediatric Department of the City of Legnano Hospital (Legnano, Italy). HIV-1 vertically infected children were classified according to Centers for Disease Control and Prevention (CDC) criteria[14] and the type of ARV therapy that they were receiving at the time of enrolment. Parents and/or the children's caregivers gave their informed consent. The study design was approved by the Departmental Committee on Ethics at San Paolo Hospital, Milan, Italy.

INTERVENTION

Bovine lactoferrin (DICOFARM, Rome, Italy) was administered orally (1 g every 8 h per day) for 6 months. This was given in addition to any existing ARV treatment.

CLINICAL MEASUREMENTS

All children underwent clinical, immune (CD4+ cell counts) and virological (plasma viral load) assessments at enrolment (T0), every 3 months during the 6 months of LF supplementation (T3, T6), and 2 months after the end of LF supplementation (T8). Data collected were compared with plasma viral load and CD4+ cell count at 3 and 6 months prior to the start of LF supplementation; therefore, six time points were considered: T – 6, T – 3, T0, T3, T6 and T8. T-lymphocyte subsets were determined by flow cytometry-triple staining (Trucount system, Becton Dickinson, FACS SORT-UK, Ltd, Oxford, UK). Plasma HIV-1 RNA samples were assayed using an Amplicor Monitor 1.5 kit (Roche Diagnostic Systems, Branchburg, NJ, USA).

STATISTICAL ANALYSIS

Descriptive statistics included mean, standard deviation (SD), median, minimum and maximum values for a number of observations. The pattern of any changes to the CD4+ cell count and plasma viral load was assessed by the analysis of variance for repeated measures or the Friedman test, when appropriate. Within-group comparisons were performed using parametric (Student's *t*-test) and non-parametric (Wilcoxon's rank sum test) analyses.

A *P*-value < 0.05 was considered statistically significant, and all of the statistical tests were two-tailed. The SPSS package version 11.5 for Windows (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis.

Results

Twenty-two HIV-1 vertically infected children entered the study consecutively from September 2002 to March 2003. The study population included nine girls and 13 boys, with a mean age of 9 years (range, 3 – 18 years). At enrolment, 18/22 patients were classified as CDC class A (light symptoms), two of 22 presented with moderate HIV-1-related symptoms (CDC class B), and two of 22 were asymptomatic (CDC class N). None of the children was classified as CDC class C. As for their immune status, 10/22 were in CDC category 1 (CD4+ cell count: ≥ 25%, no evidence of immunodeficiency) and 10/22 were in CDC category 2 (CD4+ cell count: 15 – 24%, moderate immunodeficiency); two patients belonged to CDC category 3 (CD4+ cell count < 15%, severe immunodeficiency).

At enrolment, eight of 22 subjects were not receiving ARV therapy (Group 1), 11/22 were treated with two nucleoside reverse-transcriptase inhibitors (NRTIs) or one non-nucleoside reverse-transcriptase inhibitor (NNRTI) plus one NRTI (Group 2), and three patients were treated with highly active antiretroviral therapy (HAART), i.e. a triple-therapy regimen including two NRTIs and one protease inhibitor (Group 3). The mean (± SD) age of the children when they started ARV therapy was 22 ± 19 months. None of the enrolled children changed their ARV therapy during the course of the study.

Tables 1 and 2 show the results of the evaluation of plasma viral load and CD4+ cell counts at different time points throughout the study, respectively. There were no significant changes in plasma viral load or CD4+ cell count during the 6-month pre-treatment observation period. Significant longitudinal variations in both viral

TABLE 1:
Absolute (viral copies/ml) and log_{10} plasma viral load values from 22 HIV-1 vertically infected children measured at different time points before, during and after 6 months' treatment with lactoferrin (1 g three times daily, orally)

Time point	Plasma viral load	Mean	SD	Median	Minimum	Maximum
T – 6	Copies/ml	77 778	100 630	55 000	5700	460 000
	log_{10}	4.60	0.52	4.73	3.75	5.66
T – 3	Copies/ml	84 245	93 782	68 000	2000	399 000
	log_{10}	4.61	0.60	4.83	3.30	5.60
T0	Copies/ml	78 576	85 721	26 200	1720	318 000
	log_{10}	4.54	0.65	4.41	3.23	5.50
T3	Copies/ml	41 019	55 048	14 650	1640	214 000
	log_{10}	4.28	0.55	4.15	3.21	5.33
T6	Copies/ml	41 563	46 483	19 350	1500	150 000
	log_{10}	4.28	0.60	4.26	3.17	5.17
T8	Copies/ml	68 358	83 308	23 200	677	254 000
	log_{10}	4.35	0.77	4.36	2.83	5.40

T – 6 and T – 3, 6 and 3 months prior to the start of the study, respectively; T0, baseline time point; T3 and T6, 3 and 6 months after the start of lactoferrin administration, respectively; T8, 2 months after the end of lactoferrin administration.

TABLE 2:
Absolute (cells/mm^3) and percentage CD4+ cell counts from 22 HIV-1 vertically infected children measured at different time points before, during and after 6 months' treatment with lactoferrin (1 g three times daily, orally)

Time point	CD4+ cell count	Mean	SD	Median	Minimum	Maximum
T – 6	Cells/mm^3	800	565	686	187	2280
	%	27.14	14.28	23.50	11	59
T – 3	Cells/mm^3	724	490	734	234	1932
	%	25.50	12.42	21.50	11	54
T0	Cells/mm^3	613	350	585	190	1650
	%	23.82	9.13	21.50	12	41
T3	Cells/mm^3	688	436	615	200	1890
	%	26.27	10.13	24	12	45
T6	Cells/mm^3	676	364	595	250	1492
	%	26.45	9.60	24.50	12	45
T8	Cells/mm^3	648	386	545	112	1480
	%	26.09	10.35	23.50	6	46

T – 6 and T – 3, 6 and 3 months prior to the start of the study, respectively; T0, baseline time point; T3 and T6, 3 and 6 months after the start of lactoferrin administration, respectively; T8, 2 months after the end of lactoferrin administration.

load ($P < 0.0001$) and percentage CD4+ cell count ($P = 0.005$) of the 22 patients were found during the LF treatment period, but no significant changes were found for absolute CD4+ cell count. At T8, there was no significant change in mean viral load compared with either the baseline viral load (T0) or the viral load at the end of LF administration (T6), while percentage CD4+ cell counts at T8 remained significantly higher compared with baseline (T0, $P = 0.03$).

We also investigated the changes in plasma viral load and CD4+ cell counts in Groups 1 and 2, which contained HIV-1-infected children who received no ARV therapy or those who received a combination of two antiretroviral agents, respectively. Group 3, who received HAART, could not enter the analysis because of the small number of subjects included in that group. The median percentage decrease from baseline in plasma viral load after LF treatment did not differ between the two groups (Group 1, 42%; Group 2, 49%) (Fig. 1). In contrast, the median percentage increase in CD4+ cell counts during the 6-month LF treatment period was significantly different between Groups 1 and 2 ($P = 0.02$), with a median increase of 13% in Group 1 and 27% in Group 2 (Fig. 2).

None of the patients showed new HIV-1-related symptoms during the follow-up period, and no side-effects were recorded in any patient following oral LF administration.

Discussion

We have conducted a preliminary *in vivo* study of lactoferrin supplementation in HIV-1 vertically infected children. During LF supplementation, there was a significant reduction in plasma viral load and the percentage CD4+ cell count significantly increased above baseline. Since there were no significant changes in plasma viral load and CD4+ cell counts during the

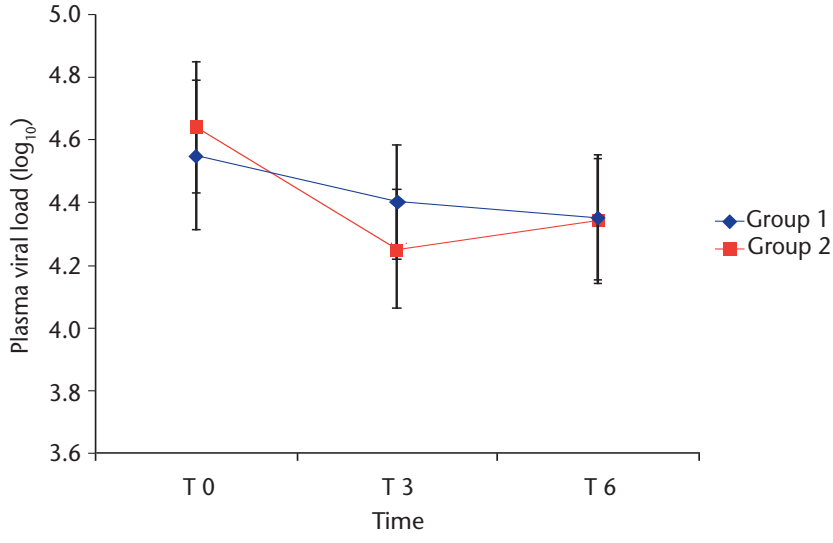

FIGURE 1: Mean plasma viral load (log_{10}) values in HIV-1 vertically infected children during 6 months of lactoferrin (1 g three times daily, orally) treatment. Group 1 (no concomitant antiretroviral therapy; *n* = 8) and Group 2 (concomitant antiretroviral therapy; *n* = 11). T0, baseline time point; T3 and T6, 3 and 6 months after the start of lactoferrin administration, respectively

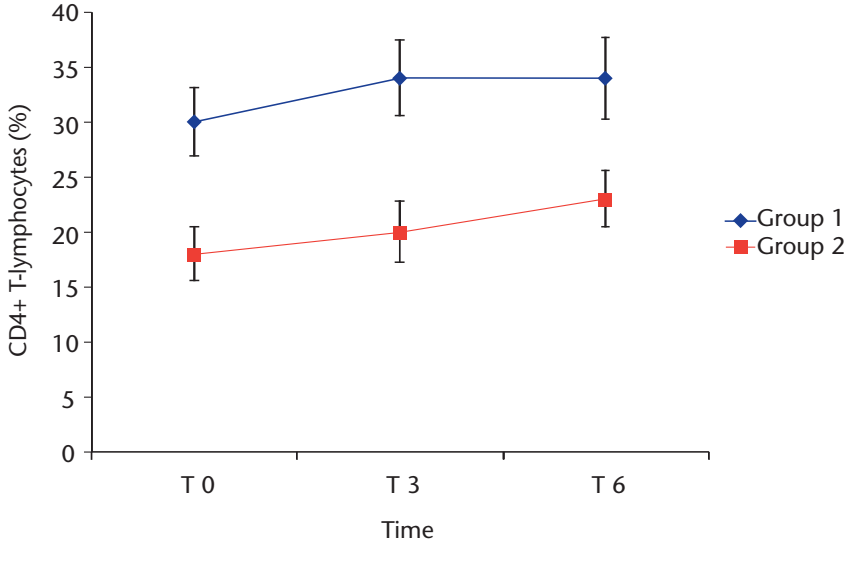

FIGURE 2: Median percentage CD4+ cell counts in HIV-1 vertically infected children during 6 months of lactoferrin (1 g three times daily, orally) treatment. Group 1 (no concomitant antiretroviral therapy; *n* = 8) and Group 2 (concomitant antiretroviral therapy; *n* = 11). T0, baseline time point; T3 and T6, 3 and 6 months after the start of lactoferrin administration, respectively

pre-treatment period, our findings suggest that the changes seen were due to oral LF supplementation rather than to any intrinsic variation in the infection status of the children in this study. The changes seen in viral load at T8 would suggest that LF needs to be administered continuously to maintain its favourable effect on plasma viral load. In contrast, the positive effect of LF on CD4+ cell counts seemed to persist even after discontinuing LF administration. However, as immune status usually parallels the changes in plasma viral load, a delayed decrease in CD4+ cell counts might be expected in the follow-up period. As the ARV therapy remained the same throughout the study period, the observed changes in viraemia and immune status were most likely due to the administration of oral LF.

As a second aim of the study, we compared the changes in plasma viral load and CD4+ cell count in ARV treatment-naïve patients and patients treated with NRTIs and/or NNRTIs. Although the decrease in plasma viral load was not significantly different between the two groups, the immune status demonstrated a greater improvement in response to LF supplementation in patients co-administered ARV therapy than in those patients not treated with concomitant ARV therapy. This finding is in agreement with the *in vitro* observations of Viani *et al.*[1] who demonstrated a synergistic effect of LF with zidovudine. Further studies are needed to differentiate between the separate effects of LF and ARV drugs on viral replication and cell invasion, and the possible synergistic mechanisms that might be involved.

We conclude that, within the limits of the present study design, our preliminary results are encouraging, particularly as LF is a natural compound produced by the human body and might be considered a 'safe' molecule without the risk of side-effects. Consequently, LF may represent a useful addition to conventional ARV therapy regimens, especially in paediatric patients, in whom conventional HIV-1 treatment might be associated with non-compliance. Larger studies, possibly based on randomized, controlled-trial designs with longer follow-up periods, are required in order to define the clinical effects and the optimal treatment schedules of LF administration.

Conflicts of interest

No conflicts of interest were declared in relation to this article.

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