Original Article

Human Immunodeficiency Virus Type-1 and cytokines in colostrum from HIV-infected mothers in Burkina Faso

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Abstract
Background: The colostrum of HIV-infected mothers contains a high number of HIV copies and is considered highly infectious. Furthermore it contains large numbers of macrophage and other mononuclear cells that are known to incorporate virus. While prevention protocols in Western countries suggest the interruption of breast feeding, at least for the first few months of life, this practice is not advisable in developing countries.
Methodology: The aim of this study was to determine the HIV load and the concentrations of IL-18, IL-16, IL-12, TGF-beta1 and TGF-beta2 in the colostrum of HIV-infected mothers living in Burkina Faso. The women all received nevirapine prophylaxis during labour.
Results: The viral load in the colostrum decreased rapidly during the first three days following delivery, while the concentration of IL-18 and IL-16 increased in the same period. IL-12, TGF-beta1 and TGF-beta2 did not show significant variations in the first three days after delivery.
Conclusions: Since the viral load decreases in the colostrum of nevirapine-treated expectant mothers, our data suggest single dose nevirapine combined with interruption of early feeding may have potential as a way to reduce the risk of MTCT.
Key Words: HIV, colostrum, Burkina Faso.

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Introduction

Human colostrum is a complex liquid produced by the mammary glands to sustain the newborn in the first days after delivery. After the first few days the composition of the mammary gland secretion changes to become a source of nutriment, but during the first 3 days after delivery the principal role of colostrum is to provide immunity to the newborn [1,2] as it contains numerous lymphocytes and macrophages. Changes that occur during this transformation include decreases in the concentration of some components, including IgE, prolactin and endorphin [3,4] and increases in the concentration of other components such as fucosyl and other oligosaccarides [5]. The concentrations of the macrophage enzyme chitotriosidase [6], as well as some cytokines (IL-1beta, IL-2, IL-6, IL-8, TNF-alpha) decrease rapidly during the first three days, suggesting that mononuclear cell numbers decrease, in conjunction with increasing concentrations of IgA and IgG [7-9] .

In mothers infected with human immunodeficiency virus (HIV), the expression of some cytokines (such as TGF-beta) in colostrum may promote HIV replication in mammary epithelial cells [10] leading to high colostral viral load and increased risk of mother to child transmission (MTCT) through breastfeeding. While in some countries HIV-infected mothers are advised not to breastfeed their infants in order to reduce this risk [11,12], however, such advice is regarded as unsuitable for mothers in developing countries because of the increased risk of infant

diarrhoea through unhygienic preparation of milk formula.

The use of single dose nevirapine, and other anti-retroviral therapies, during pregnancy has been shown to significantly reduce the risk of MTCT of HIV [13,14]. Preliminary studies have shown that the viral load of colostrum from HIV-positive mothers, treated with a single dose of nevirapine, fell within the first three days following delivery from an initial load of >100,000 copies/ml to <10,000 copies/ml [11]. Colostrum may therefore be a significant risk for MTCT of HIV and a temporary suspension of breastfeeding, for three days after birth, may significantly reduce the risk of transmission. The risk of contamination of milk is low, as milk can be safely prepared if delivery is carried out in a health-care setting. Following this temporary suspension, breastfeeding may be continued and the risk of infant diarrhoea is limited.

In this study we confirm a rapid reduction of viral load in colostrum from mothers treated with single dose nevirapine, and also report on the variations in cytokine concentrations that may affect viral load. These studies form the basis for potential interventions that may determine the efficacy of interrupted breastfeeding in prevention of MTCT of HIV.

Materials and Methods

Twenty HIV-infected mothers were recruited from the Voluntary Counseling and Testing (VCT) program at Centre Medical St Camille (CMSC), Ouagadougou, Burkina Faso, which provided ethical review and approval. Mothers with detectable viral loads were informed of the objectives of the study and following informed consent were allocated to receive Nevirapine (200 mg, single dose) during labour. Nevirapine (2mg/Kg) was administered to their babies within 72 hrs, following the recommended practice of the MTCT prevention programme at CMSC.

All of the mothers consented to the interruption of breastfeeding for the first three days after delivery, while the nurses prepared and administered a 5% glucose solution for the hydration of the newborns. Six hours after delivery, colostrum was collected by delicate vacuum pumping, using graduated sterile tubes. The colostrum samples were taken to the CMSC laboratory where the tubes were stored at -80°C.

Infants were fed formula (10 g each meal for the first day seven times a day, and increasing by 10 g each meal for the remaining two days). Colostrum was collected daily from both breasts and discarded after representative samples had been stored as above. On the fourth day the mothers were encouraged to breastfeed their babies normally.

Determination of viral load

The HIV load was measured in serial colostrum samples, transported on dry ice to the Laboratory of Virology, Ascoli-Tomaselli Hospital, Catania, Italy. After thawing, colostrum samples were centrifuged at 680 X g for 10 min at 4°C. The supernatant was removed and re-centrifuged at 10,000 X g for 30 min at 4 C° to allow removal of the lipid layer and cellular elements. Aliquots (250ul) were stored in Eppendorf tubes at -20°C. The HIV load was determined using the AMPLICOR Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) assay (Roche). The results are expressed as HIV RNA copies/ml on a log scale.

Determination of cytokines contents

Cytokine concentrations (IL-18, IL-16, IL-12, and TGF-beta 1 and 2) were determined in the same serial colostrum samples, with a commercial enzyme-linked immunosorbent assay (R&D Systems, Milan, Italy).

Statistical analysis

The non-parametric Mann-Whitney U test was used for statistical analysis.

Results

The HIV load in the colostrum of 20 HIV-infected mothers (aged 15-44 years) ranged from 8,000 to 120,000 copies/ml (mean 80,200 ± 72,600 copies/ml). By the second day the HIV load had decreased significantly (see figure 1) to a maximum of 35,000 copies/ml ($P<0.0001$), and on the third day the HIV load decreased again to below 2,000 copies/ml ($P<0.0001$).

By way of contrast, the colostral concentration of IL-18 and IL-16 increased over the first three days following delivery, though the variation was quite high.

The statistical analysis of cytokine concentration in the first three days after delivery

confirmed that only the concentrations of IL-18 and IL-16 increased significantly in the second and third days (Table 1), while the concentrations of IL-12, TGF-beta1 and TGF-beta2 remained stable over the whole period.

Table 1. Concentration (pg/ml) of IL-18, IL-16, IL-12, TGF-beta1 and TGF-beta2 in the colostrum from 20 HIV-infected mothers.

Cytokines [pg/ml, median (range)]	1° day	2° day	3° day
IL-18 (n=20)	10.67 (6-18)*	80.22 (11-257)	72.85 (10-197)
IL-16 (n=20)	74.35 (25-98)*	134.00 (37-236)	226.05 (58-487)
IL-12 (n=20)	38.35 (28-62)	31.35 (24-34)	30.65 (22-34)
TGF-beta1 (n=20)	66.97 (59-71)	82.37 (66-106)	69.42 (50-78)
TGF-beta2 (n=20)	912.05 (90-2602)	789.47 (557-835)	753.05 (436-1026)

*1° day → 2° day, P<0.05.

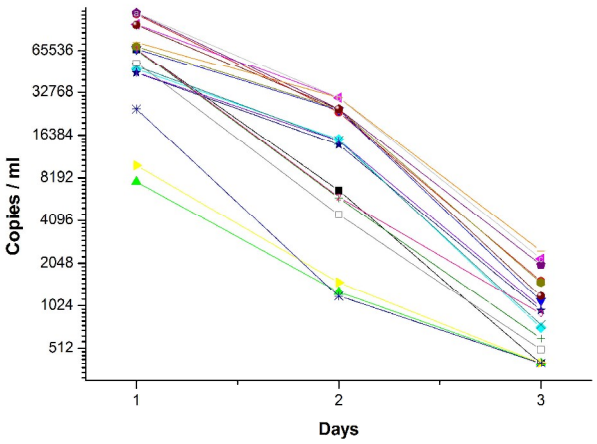

Figure 1. HIV load (copies/ml) in colostrum from 20 HIV-infected mothers.

Discussion

The results clearly show that the HIV load in the colostrum of nevirapine-treated mothers decreased significantly over the first three days following delivery, reducing the risk of MTCT in breast milk. This reduction in HIV load in colostrum could be explained as resulting from reduced replication of HIV in the presence of nevirapine. While cell counts were not done, the decrease in concentration of macrophage-derived enzymes [6] is suggestive of a progressive reduction in numbers of macrophages. Macrophages and other mononuclear cells may be an important source of HIV infection, independent of the infection derived from free HIV virions as detected by RT-PCR [15-17]. Other studies have suggested that cell-free HIV-1, which is present in mature breast milk but only at low concentrations [18] may not represent a significant source of infection in the absence of inflammation. Following treatment with nevirapine, concentrations of HIV-1 may be even lower, since nevirapine is excreted in breast milk.

Factors that influence inflammatory responses in breast milk, such as mastitis, may also promote HIV transmission through the production of HIV-infected mononuclear cells that are ingested with the milk [19-21]. Pro-inflammatory cytokines may be used as a surrogate marker of infectivity in this way. Interleukin-18, which is associated with preterm delivery and pregnancy complication [22], plays an important role in host defense in neonates. Among other effects, this cytokine stimulates expression of CD4 antigen, and so may increase the risk of HIV-1 infection. Interleukin-16 is a chemo-attractant factor found in colostrum and is responsible for the traffic of leukocytes from the maternal circulation to the breast milk [23]. Pro-inflammatory cytokines are essential in the protection of newborns from pathogens that may be transmitted in breast milk, especially in the first days of life [24], and the continued presence of IL-12 throughout the period of measurement in this study supports this view. Both IL-12 and IL-18 induce interferon-gamma (INF-gamma) secretion from T cells in a synergistic manner [25]. The presence of TGF-beta1 and TGF-beta2 may balance the increase of pro-inflammatory cytokines in breast milk [26]. Since it is not possible to directly modify the cytokines in human colostrum, we suggest that interrupting the administration of colostrum in this critical period of lactation may be advantageous.

There is now a large amount of data that shows that MTCT of HIV can be dramatically reduced by the use of antiretroviral treatment in pregnancy and by the use of single dose treatments in the perinatal period [27]. There is evidence that combination prophylaxis with nevirapine and zidovudine may reduce the risk of infection even further [28]. But there is still no

effective program, apart from avoiding breastfeeding, for the prevention of transmission in the breastfeeding period. Our data suggest single dose nevirapine combined with interruption of early feeding may reduce the risk of MTCT, since the high levels of both free virus, and of viral particles within macrophages and other mononuclear cells, can be avoided [29]. In this admittedly small study, none of the children died or acquired HIV or other infectious diseases.

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