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The effect of highly active antiretroviral treatment on viral load and antiretroviral drug levels in breast milk

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Viral load and drug levels were measured in the plasma and breast milk of nine mothers treated with highly active antiretroviral therapy (HAART) and one woman treated only with zidovudine during delivery. In all HAART-treated women after delivery the viral load was less than 400 copies/ml in plasma and breast milk. Compared with the plasma concentration, the breast milk concentration was between 68 and 90% for nevirapine, 6 and 24% for nelfinavir and 90 and 540% for indinavir.

At present little information is available concerning the degree of passage of antiretroviral drugs into human breast milk. Zidovudine, lamivudine and nevirapine are excreted into human breast milk. A single 200 mg dose of nevirapine administered to a pregnant woman resulted in a nevirapine concentration in breast milk higher than 0.1 mg/l throughout the first week (10 × *in vitro* IC_{50} against wild-type HIV) [1]. In another study, the breast milk concentration after treatment with lamivudine and zidovudine, started at week 38 of pregnancy and continued for one week throughout breastfeeding, was 0.9 mg/l (mean) for lamivudine [2]. A third study reported a maternal serum-to-breast milk ratio of 1 : 1.3 for zidovudine [3].

With regard to all other commercially available antiretroviral agents we only know that they are excreted into the milk of lactating rats. In this study we measured the viral load and antiretroviral drug levels in the plasma and breast milk of mothers treated with HAART in Belgium.

After informed consent was obtained, HIV-positive pregnant women were enrolled in the study. Women were continued on the HAART regimen prescribed by the physician responsible for their care. Women who needed HAART for their own health were continued on HAART after delivery. For women who did not need HAART for their own health (women who never had a CD4 lymphocyte count < 350 cells/μl), HAART was continued for 5 days after the first breast milk sample was obtained and was then stopped.

Nine of the 10 women enrolled in the study were treated with HAART at least during the entire second and third trimester. All women also received zidovudine intravenously (2 mg/kg per hour 3 h before delivery and then 1 mg/kg per hour until after delivery). One woman discontinued HAART during pregnancy and was treated with only zidovudine. HAART was given either during the entire pregnancy (in women 1, 2, 4 and 7) or stopped during the first trimester (women 5, 6, 8, 9 and 10). All babies received one dose of zidovudine.

During 5 days post-delivery breast milk samples were obtained using an electrical pump, just before the intake of the antiretroviral drugs and 2 h later. After 5 days the women received cabergoline 1 mg by mouth to stop breast milk production. None of the children were breastfed.

The viral load was tested on plasma and breast milk samples obtained just before the intake of the antiretroviral drugs, and one, 3 and 5 days after delivery. The Ampliprep Cobas Amplicor HIV-1 Monitor test (version 1.5; Roche Diagnostic Systems Inc., Branchburg, New Jersey, USA) with a lower detection limit of 50 copies/ml was used. Centrifugation of the breast milk was performed (7 min at 1300***g***) to spin down the cells. The viral load was measured in the supernatant.

Nevirapine [4] and protease inhibitor [5] drug levels in the plasma were measured at the Department of Clinical Pharmacology, UMC, Nijmegen, the Netherlands, by high-performance liquid chromatography (HPLC) as described earlier [4,5]. No drug levels were measured for nucleoside analogues. For the different antiretroviral drug levels in breast milk an adapted reversed-phase HPLC (buffer concentration, temperature, ultraviolet wave length) was used. Before analysis, the mother's milk was warmed up until a temperature of 37°C was reached in order to obtain a homogeneous mixture. Samples were filtered using the syringe filter from Waters, 4 mm nylon, 0.45 μm.

The correlation between plasma and breast milk was determined using the Pearson's correlation coefficient. Calculations were performed using SPSS version 9.0 (SPSS Inc., Chicago, Illinois, USA).

The study was approved by the committee for medical ethics of the Institute of Tropical Medicine, Antwerp, Belgium.

Ten women participated in the study. The characteristics of these women are shown in Table 1. They were all infected heterosexually. Half of the women already had children. None of the women had signs of breast milk inflammation.

In eight of the nine women treated with HAART the viral load was less than 50 copies/ml in plasma and breast milk (Table 1). In only one HAART-treated woman a low but detectable viral load (172 copies/ml) was noted only in the colostrum. She had stopped taking HAART for 2 weeks when she was in her eighth month of pregnancy. In the woman only treated with zidovudine intravenously during delivery, the viral load was detectable in all plasma and breast milk samples.

For nevirapine, 34 plasma and breast milk samples taken at the same moment (paired samples) were available for analysis. Compared with the plasma concentration, the breast milk concentration of these drugs was between 68 and 90% for nevirapine. The correlation of plasma/milk drug concentration was statistically significant (Pearson coefficient 0.68, $P < 0.001$).

For nelfinavir, 17 plasma and breast milk samples taken at the same moment (paired samples) were available for analysis. Penetration of nelfinavir into the milk from plasma was 6–24%. The correlation plasma/milk drug concentration was also statistically significant (Pearson coefficient 0.70, $P < 0.001$). The nelfinavir metabolite M8 was not detected in breast milk. Indinavir seems to penetrate well in breast milk, but only a small number of samples were examined.

This study suggests that when HAART is started sufficiently early during pregnancy and is continued during breastfeeding the viral load in breast milk in most of the patients will decrease to below detectable levels. We only measured the viral load in the breast milk of participating women during the first week after delivery, but it has been shown that the viral load in this period is the highest [6].

Moreover, this study confirms that not only nevirapine, but also the protease inhibitors nelfinavir and indinavir can be detected in breast milk. For the latter two this has never been documented so far. The degree of passage of antiretroviral drugs in breast milk, however, may be different. Compared with the plasma concentration, the breast milk concentration was between 68 and 90% for nevirapine, 6 and 24% for nelfinavir and 90 and 540% for indinavir.

Nevirapine and nelfinavir were present in the breast milk in a sub-therapeutic concentration when regarded as

Table 1. Characteristics of the participants.

Participant	Age (years)	GO	HAART regimen	CS	CD4 cell nadir	CD4 cell count before delivery	Plasma VL d1	Colostrum VL	Plasma VL	BM VL
1	32	A	RTV 200 mg bid IDV 600 mg bid 3TC 150 mg bid D4T 40 mg bid	Yes	209	445	< 50	172	< 50 (d3)	ND***
2	26	A	ZDV 300 mg bid 3TC 150 mg bid NFV 1250 mg bid	Yes	166	596	< 50	< 50	ND	< 50 (d4)
3	32	A	No HAART	Yes	208	288	96	261	78 (d3)	441 (d3)
4	21	A	D4T 40 mg bid 3TC 150 mg bid NFV 1250 mg bid	Yes	278	320	< 50	< 50	< 50 (d5)	< 50 (d5)
5	32	A	ZDV 300 mg bid ddI 400 mg qd NFV 1250 mg bid	Yes	293	357	< 50	< 50	< 50 (d4)	< 50 (d4)
6	29	A	ZDV 300 mg bid 3TC 150 mg bid NFV 1250 mg bid	No	484	795	< 50	< 50	< 50 (d3)	< 50 (d3)
7	29	A	NVP 200 mg bid 3TC 150 mg bid ZDV 300 mg bid	Yes	354	742	< 50	< 50	< 50 (d5)	< 50 (d5)
8	22	A	NVP 200 mg bid 3TC 150 mg bid ZDV 300 mg bid	Yes	215	448	< 50	70	< 50 (d5)	< 50 (d5)
9	23	A	ZDV 300 mg bid 3TC 150 mg bid NFV 1250 mg bid	Yes	922	922	< 50	< 50	< 50 (d5)	< 50 (d5)
10	26	T	NVP 200 mg bid D4T 30 mg bid 3TC 150 mg bid	Yes	344	652	< 50	< 50	< 50 (d5)	< 50 (d5)

BM, Breast milk; CS, caesarean section; d, day (d1, day colostrum was obtained); ddI, didanosine; D4T, stavudine; GO, geographical origin: A, Africa, T, Thailand; HAART, highly active antiretroviral therapy; IDV, indinavir; NFV, nelfinavir; NVP, nevirapine; RTV, ritonavir; 3TC, lamivudine; VL, viral load; ZDV, zidovudine. All participants also received zidovudine intravenously during labour: bid, twice daily, qd, once daily.

treatment for established HIV infection. Whether these drug levels would be sufficient to have a prophylactic effect remains to be investigated.

The results of this study need to be interpreted with caution. HPLC measures the total amount of a drug in the serum or breast milk. In blood, the percentage of the drug bound to proteins and the percentage free drug (the active fraction) is well known. In plasma, nevirapine is 55% protein bound and for nelfinavir the figure is 98–99%. On the other hand, in breast milk we do not know the fraction of antiretroviral drugs that is protein bound. Another unknown is how the neonate will absorb and metabolize the antiretroviral drugs present in the breast milk. Although exposure to antiretroviral drugs through their mother's milk may protect infants, those who are infected *in utero* could be at risk of developing resistance mutations as a result of sub-therapeutic drug levels. The different degree of passage of antiretroviral drugs in breast milk could potentially lead to the transmission of resistant viruses during breastfeeding, particularly if mothers do not adhere well to their antiretroviral regimen.

Studies in countries with limited resources, including a larger number of mother–children pairs, measuring the viral load (and eventually resistance testing) and drug levels in breast milk and plasma, will be needed to identify those HAART regimens that are most likely to be successful in decreasing the viral load in breast milk without potential toxicity or other harm for the child. Such studies could be performed with little additional cost as part of the prevention of mother to child transmission plus programmes using different antiretroviral regimens. Regimens identified by this process should then be further evaluated in randomized clinical trials.

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Evidence of activity of Irinotecan in patients with advanced AIDS-related Kaposi's sarcoma

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Fourteen HIV-infected patients with advanced Kaposi's sarcoma (KS) received Irinotecan 150 mg/m^2 intravenously on days 1 and 10. All patients were relapsed/progressed during highly active antiretroviral therapy, administered as primary antineoplastic therapy. An objective response, all partial remissions, occurred in 75% of patients. Irinotecan was well tolerated, severe leukopenia occurred in only 33% of patients. In HIV-infected patients with advanced KS, irinotecan is active and well tolerated.

The profile of AIDS Kaposi's sarcoma (KS) has favourably changed since the widespread use of highly active antiretroviral therapy (HAART) [1–5]. In patients with rapidly progressive cutaneous disease, widespread symptomatic disease or visceral involvement, cytotoxic chemotherapy remains the most successful modality of therapy. However, to date, there is no curative treatment for advanced-stage KS [6,7].

Irinotecan (CPT-11; Camptosar), a semi-synthetic camptothecin derivative, is a topoisomerase I inhibitor with a broad spectrum of activity against solid tumours [8]. Preclinical studies indicate that camptothecines are most likely cytotoxic against two tumour compartments: in addition to tumour cells of epithelial origin, the drugs act against endothelial cells and prevent the growth of tumour microvessels. Irinotecan showed a significant reduction in neoangiogenesis in mouse cornea model. Unlike other camptothecin analogues, the drug had acceptable toxicity in this preclinical study [9].

This observation has led to the suggestion that Irinotecan might have activity in KS, an angioproliferative tumour characterized by angiogenesis, endothelial spindle cell growth other than inflammatory cell infiltration and oedema [10]. During the past 9 years, Irinotecan has undergone clinical evaluation in a number of malignancies, and is accepted as an antineoplastic drug in colorectal cancer, but to our knowledge it has not been evaluated in epidemic KS [8,11].

We here report preliminary data on a phase II study with Irinotecan in HIV-infected patients with advanced KS who had experienced neoplastic progression during HAART.

From June 1999 to December 2004, within the Italian Cooperative Group on AIDS and Tumors (GICAT), we treated 14 patients with disseminated epidemic KS with Irinotecan 150 mg/m^2 given intravenously on days 1 and 10. The administration of chemotherapy was repeated every 21 days until progression. Eligible patients had to have relapsed/progressed during HAART, administered as primary antineoplastic therapy. Granulocyte colony-stimulating factor (G-CSF) was given subcutaneously at the dose of 5 μg/kg per day beginning on day 2, at the discretion of the treating physician, to patients experiencing severe granulocytopenia in the previous cycle. Oral alkalinization with sodium bicarbonate, magnesium oxide, neutral/basic water, and ursodeoxycholic acid on days 2–5 and the control of defecation was performed to reduce Irinotecan-induced side-effects, as already reported [12]. Assessments of toxicity and response were performed according to the World Health Organization and AIDS Clinical Trials Group criteria, respectively. Patients' characteristics, previous treatments, and results are shown on Table 1.

All patients were men, eight patients (57%) had received previous anthracycline or taxane-based chemotherapy, and five patients had received more than one previous chemotherapy regimen. Eleven patients (79%) received lopinavir–ritonavir-based HAART concomitantly with