A placebo-controlled trial of didanosine plus stavudine, with and without hydroxyurea, for HIV infection

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Objective: To explore the short-term effects on surrogate markers for HIV progression of didanosine (ddI) plus stavudine (d4T), with or without hydroxyurea.

Design: Randomized, double-blinded, prospective study.

Setting: Swiss HIV Cohort Study.

Patients: A total of 144 patients (75% antiretroviral-naive) were studied (mean baseline HIV-1 RNA, 4.53 $\log_{10}$ copies/ml; mean CD4 cell count, 370×10^6/l).

Intervention: Patients received ddI (200 mg twice daily) plus d4T (40 mg twice daily), with additional hydroxyurea (500 mg twice daily) or placebo.

Main outcome measures: The primary endpoint was a reduction of viraemia below 200 copies/ml after 12 weeks. At that time, patients who did not reach the primary endpoint were withdrawn in the hydroxyurea arm, whereas patients in the placebo group had the option of adding hydroxyurea to ddI and d4T. All patients were followed until week 24.

Results: After 12 weeks, 54% of the patients randomized to hydroxyurea had viraemia below 200 copies/ml, compared with 28% on placebo ($P < 0.001$). Using an ultrasensitive assay with a limit of detection of 20 copies/ml, 19% of patients receiving hydroxyurea had viraemia levels below 20 copies/ml, compared with 8% on placebo ($P = 0.05$). Mean decrease in HIV-1 RNA was 2.3 and 1.7 $\log_{10}$ copies/ml for hydroxyurea and placebo groups, respectively ($P = 0.001$). Hydroxyurea was found to induce lymphopenia (-124×10^6/l). Increase in CD4 cell counts was $+28 \times 10^6$/l during hydroxyurea treatment compared with $+107 \times 10^6$/l on placebo ($P = 0.001$).

Conclusions: Hydroxyurea improved the antiviral activity of d4T and ddI over a 12-week period, but was associated with a smaller increase in CD4 cell counts due to hydroxyurea-induced lymphopenia.

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Introduction

Large randomized trials have shown the clinical benefit of combined therapies [1–4]. However, the costs and the complicated dosing schedules of regimens including protease inhibitors raise issues of equity and compliance.

Didanosine (ddI) and stavudine (d4T) have synergistic antiviral activity *in vitro* and *in vivo* [5,6]. The synergy between ddI and hydroxyurea resulted in the suppression of viral production in resting lymphocytes and activated peripheral blood mononuclear cells (PBMC) [7–9]. *In vivo*, the ddI–hydroxyurea combination was associated with a sustained decrease in HIV viraemia [10,11]. Two patients with high CD4 cell counts and low baseline levels of viraemia were treated with ddI plus hydroxyurea and did not experience a viral rebound after the interruption of treatment [12], suggesting that hydroxyurea may play an important role for the long-term control of HIV infection.

Published clinical data on the use of hydroxyurea are mostly anecdotal. Nevertheless, hydroxyurea in combination with ddI and d4T has become one of the most frequently prescribed antiretroviral drug combinations [13].

We aimed to determine the short-term effect of the combination of ddI plus d4T, with or without hydroxyurea, on surrogate markers of progression in HIV-infected individuals with moderate immunosuppression.

Patients and methods

HIV-1-positive patients from seven hospitals participating in the Swiss HIV Cohort Study [14] with CD4 cell counts of $200–500 \times 10^6$/l were randomly allocated to receive ddI (200 mg twice daily), plus d4T (40 mg twice daily if body weight > 50 kg, 30 mg twice daily if body weight < 50 kg), plus hydroxyurea (500 mg twice daily) or placebo. The primary endpoint was to compare the proportion of patients who reached 200 HIV RNA copies/ml at week 12, with a 24-week follow-up.

Major inclusion criteria were two consecutive CD4 cell counts between 200 and 500×10^6/l and two HIV-1 RNA plasma levels above 1000 copies/ml, no prior use of d4T and hydroxyurea, and age 20 years or older. ddI treatment of less than 6 months' duration was permitted. Patients with a history of pancreatitis, peripheral neuropathy or chronic alcohol use were excluded. The protocol was approved by the Swiss National Regulatory Office and by each institution's ethics committee. Participants gave written informed consent. Patients were assessed twice before enrolment and at weeks 4 and 12 (clinical evaluation, safety laboratory tests including complete blood counts, liver and pancreatic tests, CD4 and CD8 cell counts, and HIV-1 RNA determination). Adverse events were graded according to AIDS Clinical Trials Group criteria, and clinical events defined according to the Centers for Disease Control and Prevention 1993 classification. All data were prospectively recorded.

The randomization code was broken at week 12 and treatment was continued according to the HIV-1 RNA level: patients who reached the primary endpoint (responders) continued the same treatment, whereas non-responders (HIV-1 RNA > 200 copies/ml) were withdrawn (hydroxyurea group), or hydroxyurea was optionally added to ddI plus d4T (placebo group). These rules were established in compliance with the current Swiss guidelines for the treatment of HIV infection [15].

HIV-1 RNA level was quantified using the Amplicor Monitor assay (Roche, Basel, Switzerland). Plasma of patients who achieved a reduction of viraemia below 200 copies/ml were retested using a modified assay with a limit of detection of 20 copies/ml [16].

All analyses were performed on an intent-to-treat basis for the blinded part of the trial, and on an on-treatment basis for the non-blinded part of the trial. Statistical methods for group comparisons included χ^2 tests for proportion and t tests for the continuous variables. Subgroup analysis was planned to compare treatment effectiveness among patients with or without prior antiretroviral exposure, as well as according to initial levels of HIV-1 RNA (< 4.0 $\log_{10}$ copies/ml, 4.0–5.0 $\log_{10}$ copies/ml, > 5.0 $\log_{10}$ copies/ml).

Compliance was evaluated by a questionnaire to the treating physician and the study nurse of each patient. Compliance was considered good if these evaluations suggested that the patient missed less than one dose every week.

Results

A total of 145 patients were randomly assigned to the study. The baseline characteristics of the study population are shown in Table 1. Treatment arms were well balanced.

Ten (7%) patients could not be evaluated at week 12 and were considered non-responders for the intent-to-treat analysis. At week 12, 37 non-responders (18 in the placebo group, and 19 in the hydroxyurea group) chose another treatment as a result of a poor response to the initial treatment. Thirty-four responders (87%) initially randomized to hydroxyurea continued the triple combination. Nineteen responders (95%) in the

Table 1. Baseline characteristics of patients.

	ddI + d4T + hydroxyurea (n = 72)	ddI + d4T + placebo (n = 72)
Mean ± SE HIV-1 RNA (log_{10} copies/ml)	4.51 ± 0.07	4.54 ± 0.07
Mean ± SE CD4 cells ($\times 10^6$/l)	375 ± 14	364 ± 14
Mean ± SE CD8 cells ($\times 10^6$/l)	953 ± 46	1085 ± 69
Previous antiretroviral treatments (%)		
None	76	74
Zidovudine experienced	22	25
ddI (< 6 months)	7	12
Zalcitabine	8	7
Lamivudine	1	1
Saquinavir	3	3

None of the differences were statistically significant. ddI, Didanosine; d4T, stavudine.

placebo arm continued on ddI plus d4T. Hydroxyurea was added to ddI plus d4T in 24 non-responders (49%) initially randomized to placebo (Fig. 1).

Week 12

Hydroxyurea resulted in a greater proportion of patients with viraemia below 200 and 20 copies/ml (Table 2). In subgroup analyses, the proportion of patients with HIV-1 RNA < 200 copies/ml was higher in antiretroviral-naive patients [58% (32 out of 55) on hydroxyurea, 32% (17 out of 53) on placebo] than in antiretroviral-experienced patients [41% (23 out of 55) on hydroxyurea, 16% (nine out of 53) on placebo; $P > 0.2$]. When stratified according to initial HIV-1 RNA level, the proportion of patients with HIV-1 RNA < 200 copies/ml was not statistically different at week 12 in both groups for patients with viraemia < 4.0 log_{10} copies/ml at inclusion (n = 21). In patients with an intermediate initial HIV-1 RNA level (4.0–5.0 log_{10} copies/ml), 50% (22 out of 44) in the hydroxyurea and 28% (13 out of 47) in the placebo group had HIV-1 RNA < 200 copies/ml at week 12 ($P < 0.03$). No patient (out of 15) with HIV-1 RNA > 5.0 log_{10} copies/ml reached < 200 copies/ml in the placebo group, compared with 47% (eight out of 17) receiving hydroxyurea ($P = 0.002$).

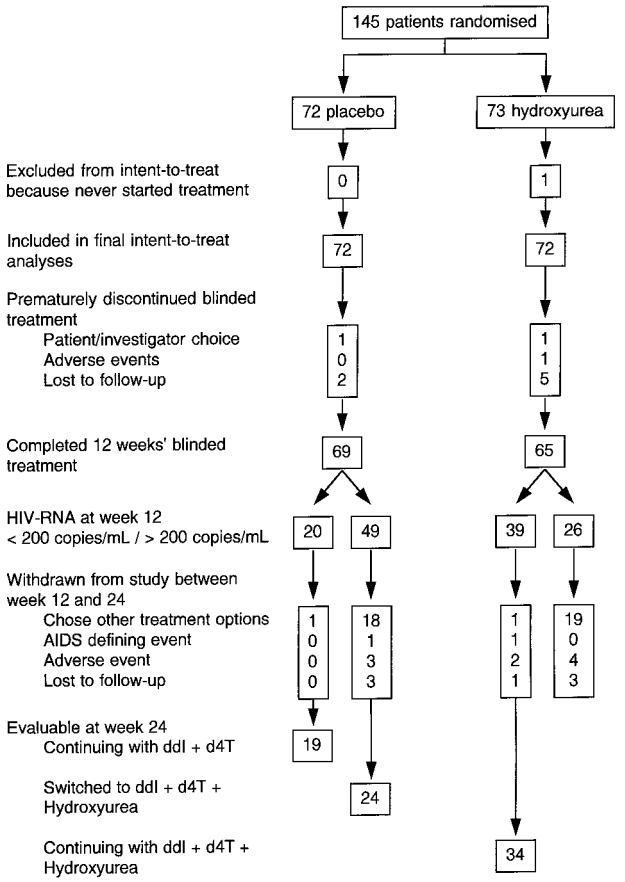

Fig. 1. Trial profile. d4T, Stavudine; ddI, didanosine.

Table 2. Primary endpoints at week 12: proportion of patients with HIV-1 RNA < 200 copies/ml (standard Roche assay) and < 20 copies/ml (ultrasensitive assay), by intent-to-treat analysis.

Parameter	No. patients/total (%) Week 4	Week 12	P*
HIV-1 RNA < 200 copies/ml			
Placebo	19/72 (26)	20/72 (28)	
Hydroxyurea	22/72 (31)	39/72 (54)	0.001
HIV-1 RNA < 20 copies/ml			
Placebo	ND	6/72 (8)	
Hydroxyurea	ND	14/72 (19)	0.05

*χ^2 test at week 12, placebo versus hydroxyurea. ND, Not done.

Decreases in HIV-1 RNA at weeks 4 and 12 were more profound in patients randomized to hydroxyurea (Table 3). In contrast, the increase in CD4 cell counts was less than that with hydroxyurea, despite a similar increase in CD4/CD8 ratio and CD4 cell percentage in both groups (Table 3), because of hydroxyurea-induced lymphopenia ($-124 \pm 54 \times 10^6$/l).

Table 3. Evolution of HIV-1 RNA, CD4 and CD8 cell counts at week 4 and 12.

Parameter	Mean ± SE change Week 4	Week 12	P*
HIV-1 RNA (log_{10} copies/ml)			
Limit of detection of 200 copies/ml			
Placebo	–1.6 ± 0.1	–1.5 ± 0.1	
Hydroxyurea	–1.6 ± 0.1	–1.9 ± 0.1	0.01
Limit of detection of 20 copies/ml			
Placebo	ND	–1.7 ± 0.1	
Hydroxyurea	ND	–2.3 ± 0.1	0.001
CD4 cells ($\times 10^6$/l)			
Placebo	+102 ± 15	+38 ± 12	
Hydroxyurea	+107 ± 18	+28 ± 13	0.001
CD8 cells ($\times 10^6$/l)			
Placebo	+61 ± 38	+5 ± 50	
Hydroxyurea	–28 ± 31	–124 ± 32	0.03
CD4/CD8 ratio			
Placebo	+0.05 ± 0.02	+0.09 ± 0.02	
Hydroxyurea	+0.07 ± 0.02	+0.11 ± 0.02	0.4

*Student's t test at week 12, placebo versus hydroxyurea. ND, Not done.

Table 4. Changes in HIV-1 RNA, CD4 and CD8 cells counts at week 12 and 24, according to open-label treatment after week 12.

Randomized treatment (baseline to week 12)	Open treatment after week 12	n	Mean ± SE HIV-1 RNA changes (log_{10} copies/ml)*			Mean ± SE CD4 cells ($\times 10^6$/l)		
			Week 12	Week 24	$P^\dagger$	Week 12	Week 24	$P^\dagger$
ddI + d4T + hydroxyurea	ddI + D4T + hydroxyurea	34	−2.8 ± 0.1	−2.6 ± 0.2	0.1	+36 ± 16	+31 ± 17	0.8
ddI + d4T + placebo	ddI + D4T + hydroxyurea	24	−1.1 ± 0.2	−1.9 ± 0.1	< 0.0001	+123 ± 25	+86 ± 26	0.1
ddI + d4T + placebo	ddI + D4T	19	−2.5 ± 0.1	−2.3 ± 0.1	0.2	+60 ± 35	+73 ± 35	0.5

*Limit of detection, 1.3 log_{10} copies/ml. †Student's t-test, week 12 versus week 24. ddI, Didanosine; d4T, stavudine.

When stratified according to previous antiretroviral treatment, the mean HIV-1 RNA change (limit of detection, 200 copies/ml) at week 12 for naive patients was -2.0 ± 0.1 log_{10} and -1.8 ± 0.1 log_{10} copies/ml for the hydroxyurea and placebo groups, respectively, compared with -1.4 ± 0.3 log_{10} and -0.9 ± 0.2 log_{10} copies/ml for experienced patients ($P < 0.02$).

Week 24

A total of 79% (27 out of 34) of patients who continued hydroxyurea had persistent levels of HIV-1 RNA < 200 copies/ml, and 17 (63%) had < 20 HIV-1 RNA copies/ml. A rebound of HIV-1 RNA was observed in seven patients, with a mean ± SE increase of 1.1 ± 0.2 log_{10} copies/ml. One patient had stopped the treatment before week 24 and four reported poor compliance (Table 4).

Nineteen patients continued ddI plus d4T after week 12. Fifteen (79%) of these patients maintained HIV-1 RNA < 200 copies/ml at week 24, and five (33%) had < 20 HIV-1 RNA copies/ml. A HIV-1 RNA rebound was observed in four patients, with a mean ± SE increase of 0.7 ± 0.1 log_{10} copies/ml. Two patients reported poor compliance.

Hydroxyurea was added to ddI plus d4T in 24 non-responders. At week 24, eight (33%) patients had < 200 HIV-1 RNA copies/ml (one with HIV-1 RNA < 20 copies/ml). A significant additional mean ± SE decrease of 0.8 ± 0.2 log_{10} HIV-1 RNA copies/ml was observed when compared with week 12 ($P < 0.001$). The increase in CD4 and CD8 cell counts observed at week 12 was stable at week 24.

Table 5. Clinical and laboratory adverse events during 24 weeks of follow-up.

	Placebo	Hydroxyurea	P*
Clinical adverse events	25	36	0.06
Abdominal pain	4	5	0.7
Cutaneous rash	4	5	0.7
Diarrhoea	9	15	0.2
Fatigue	2	10	0.02
Headache	7	2	0.09
Nausea/vomiting	7	11	0.3
Neuropathy			
Grade 1	6	9	0.4
Grade 2	4	6	0.5
Grade 3	0	3	0.08
All grades	10	18	0.09
Laboratory abnormalities			
Neutropenia			
Grade 1	3	11	0.04
Grade 2	0	2	0.2
Grade 3	0	1	0.3
Thrombopenia			
Grade 1	1	7	0.03
Grade 2	2	0	0.2
Grade 3	0	1	0.3
Elevated lipase	3	5	0.5
Elevated ALT/AST	34	36	0.7

*χ^2 test, placebo versus hydroxyurea. ALT, Alanine aminotransferase; AST, aspartate aminotransferase.

Adverse events

Clinical adverse events were slightly more common among hydroxyurea-treated patients, although only fatigue was significantly more frequent. A trend in more frequent neuropathies was observed in the hydroxyurea group. All side-effects resolved when the treatment was discontinued. Neutropenia and thrombopenia were more frequent with hydroxyurea but resolved at discontinuation of hydroxyurea (Table 5).

Twenty-three stage B HIV-related illnesses occurred during the trial, 12 in the hydroxyurea group and 13 in the placebo group. One patient in each group presented with a new diagnosis of Kaposi's sarcoma.

Discussion

The combination of ddI, d4T and hydroxyurea is one of the most popular antiretroviral treatment combinations. According to a recent telephone poll of prescribers, this combination was the fifth most frequent choice for initial treatment, being mentioned by 11% of prescribers, compared with 17% for zidovudine, lamivudine plus indinavir, or d4T, lamivudine plus nelfinavir [13]. Despite its popularity, published data on this combination are scarce. Our prospective, double-blind, randomized study provides evidence for the short-term benefit of added hydroxyurea as measured by viraemia after 3 months, and quantifies short-term toxicity.

The clinical benefits of reverse transcriptase inhibitor combinations have been shown within and outside large randomized trials [1,2,17,18]. These trials established the prognostic value of the initial reduction of viraemia following antiviral treatment [17,19,20]. In the present study, both combinations resulted in a drop of viraemia greater than 1.7 $\log_{10}$ copies/ml. All compliant patients with low viraemia at baseline (< 4.0 $\log_{10}$ copies/ml) had viraemia below 200 copies/ml at week 12. The antiviral efficacy of hydroxyurea clearly appeared in patients with more than 4.0 $\log_{10}$ HIV-1 RNA copies/ml at baseline; in this group, one in two patients receiving hydroxyurea reached HIV RNA values below 200 copies/ml, compared with only one in five in the placebo group. The proportion of patients with undetectable HIV-1 RNA was even higher (64%) in compliant patients. In addition, only patients treated with hydroxyurea had HIV-1 RNA below 20 copies/ml at week 12. Pretreatment with ddI was associated with a smaller decrease in HIV-1 RNA and with a smaller proportion of patients with undetectable viraemia.

In contrast, CD4 cells increased only marginally during hydroxyurea therapy because of hydroxyurea-induced lymphopenia, as previously described [21]. In patients with no or only minimal immunosuppression, the effect on viraemia appears to be paramount when judging a new treatment, because the decrease of viraemia after initiation of antiviral therapy is a stronger predictor of the risk of clinical progression than changes in the CD4 cell counts [17]. In contrast, the lack of effect of ddI–d4T plus hydroxyurea on CD4 cell counts should discourage physicians from using this combination in patients with advanced immunosuppression where restoration of immune function is most important. Definite answers to the question of the clinical impact of the effects of hydroxyurea on CD4 cell counts could only be obtained by a prospective trial with clinical endpoints.

According to recent published principles guiding drug choice for antiretroviral therapy [22], ddI–d4T plus hydroxyurea therapy may be an attractive combination for the following reasons. First, hydroxyurea is convenient and cheap compared with protease inhibitors. It requires one pill twice daily, and the combination with ddI and d4T was well tolerated in the majority of the patients. Contrary to experience with protease inhibitors, no unexpected interactions with concomitant medications were observed. Although the tolerance was usually good, a trend towards more adverse events was noted with hydroxyurea. Bone-marrow toxicity is a well-known side-effect of hydroxyurea and rapidly resolved when the drug was stopped. A trend towards more frequent neuropathies was also observed.

Second, hydroxyurea plus ddI–d4T resulted in a 1.9 $\log_{10}$ decrease in HIV RNA (2.3 $\log_{10}$ reduction when an assay with a limit of detection of 20 copies/ml was used). In comparison, a decrease of viraemia of 1.1 $\log_{10}$ after 12 weeks of treatment was observed in patients treated with zidovudine and lamivudine [23], and 1.3–1.7 $\log_{10}$ with the combination of ddI and hydroxyurea [10,24]. HIV RNA reductions above 2.0 $\log_{10}$ were only achieved when protease inhibitors were combined with reverse transcriptase inhibitors [3,4]. Even with the combination of zidovudine, lamivudine and indinavir, fewer than one-third of the patients had viraemia < 1.7 $\log_{10}$ copies/ml after 12 weeks of treatment, and the maximal decrease in viraemia was achieved after 24 weeks of treatment [3,4,23]. Thus, direct comparison with our results is difficult. The potential to decrease viraemia below detectable levels may have been underestimated in our study, due to the predefined study endpoint at week 12. The relatively short follow-up was a necessary compromise in view of the widespread availability of protease inhibitors in Switzerland.

Third, because d4T and hydroxyurea readily cross the blood–brain barrier, both drugs should be potentially effective in the central nervous system [7,25]. Finally, the development of resistant mutants might be a minor concern than with non-nucleoside reverse transcriptase inhibitors, lamivudine or protease inhibitors [26–29]. The emergence of drug-resistant strains to d4T and ddI is infrequent when both drugs are combined [5,30,31]. Hydroxyurea targets the cellular enzyme ribonucleotide reductase, which is not expected to mutate. Although hydroxyurea, when combined with ddI, did not prevent the emergence of the ddI codon 74 mutation, the codon 74 mutants remained sensitive to standard doses of ddI when used in combination with hydroxyurea *in vitro* [32,33]. Despite these reassuring data, it is not clear how the codon 74 mutation will impact on the effectiveness of the ddI plus hydroxyurea combination *in vivo* over the long term.

In conclusion, the combination of ddI, d4T and hydroxyurea has potent antiviral activity and might be of interest in HIV-infected patients with moderate immunosuppression and low-to-intermediate levels of viraemia. The low cost and good tolerance of hydroxyurea makes it an attractive candidate for combined therapies, but the modest increase in CD4 cell counts and the trend to more adverse events inspire caution.

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Appendix

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