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Short communication

Pilot clinical trial of the combination of hydroxyurea and didanosine in HIV-1 infected individuals

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We previously reported the synergistic effect of the combination of compounds of the hydroxamate family [D-aspartic acid β-hydroxamate or hydroxyurea (HU)] with didanosine (ddI) in infected resting lymphocytes in vitro Malley et al., 1994a, resulting in the total suppression of viral production with no effect on the cells' ability to replicate normally after treatment Malley et al., 1994b. This suppressive effect has also been observed in actively replicating lymphocytes and in monocyte-macrophages (unpublished data). The synergy of HU with ddI in vitro was subsequently confirmed by other authors Lori et al., 1994. We have now recently shown in a pilot trial that the combination of HU and ddI can induce a large reduction in viral load in the peripheral blood of HIV-1 infected individuals down to non-detectable levels in half the cases studied Biron et al., 1995.

We report here some aspects of this pilot clinical trial of the combination of HU and ddI in HIV-1 infected individuals. The study was approved by a Government-approved ethics committee in France. All patients gave their informed consent. The objectives of the trial were, firstly, to evaluate the potential side-effects of this drug combination and, secondly, to evaluate changes in CD4 count and in viral load as measured by the quantitation of infectious virus in PBMC and plasma viral RNA, after 3 months' treatment. Twelve HIV-1 seropositive subjects, nine male and three female, age range 26–60, with CD4 counts between 263 and 582/mm^3, asymptomatic, previously untreated, and with a hematological profile corresponding to grade 0 of the WHO classification (WHO offset publication, 48, 1979), were included. Female participants agreed to use effective contraception. The treatment protocol was 90 days, with 200 mg ddI twice daily and 500 mg HU twice daily.

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Table 1
Virological and immunological data at baseline and after 3 months' treatment with a combination of didanosine and hydroxyurea

Patient	CD4+ Cells/mm^3 (%)		PCR RNA RNA copies/ml plasma		Detection of infectious virus PBMC (TCID50/10^6 cells)	
	Day 0	Day 90	Day 0	Day 90	Day 0	Day 90
1	470 (16)	401 (22)	128973	156	234.2	3.5
2	365 (13)	584 (23)	30273	1308	61.6	1.8
3	311 (20)	373 (36)	45580	559	42.5	<1
4	319 (14)	412 (16)	12773	404	19.0	<1
5	321 (36)	364 (32)	115356	882	3.3	0
6	427 (32)	557 (37)	43129	1591	1	0
7	318 (25)	575 (36)	4375	Non det	55.9	<1
8	582 (22)	813 (35)	9973	Non det	0	0
9	560 (28)	1030 (34)	11091	Non det	<1	0
10	295 (27)	872 (41)	3521	Non det	<1	0
11	263 (14)	374 (22)	12713	Non det	7.2	0
12	474 (24)	526 (27)	13324	Non det	<1	0

Non det, non detectable. (%) = CD4+ cells as a percentage of total lymphocytes. Infectious HIV in PBMC was quantified by determining the tissue culture 50 percent infective doses ($TCID_{50}$) using a p24 Ag assay as described elsewhere (Rouzioux et al., 1992). Plasma viral RNA levels were quantified by polymerase chain reaction (PCR) using the ultrasensitive PCR Amplicor HIV Monitor (Roche Diagnostic Systems, Branchburg, NJ, USA) (sensitivity threshold = 200 RNA copies/ml).

Overall tolerance was good. No patient interrupted treatment due to clinical or biological side-effects. An increase in mean corpuscular volume was observed in all patients with an average volume at day 90 of 102.2 fl. No changes in red blood cells, white blood cells, platelets, hemoglobin, amylases, lipases or transaminases were noted. This good tolerance was accompanied by a substantial decrease in viral load. Infectious virus was recovered from PBMC in all patients at day 0, with titers ranging from <1 to 234.2 $TCID_{50}$ (Table 1): for patient 8 in vitro PBMC activation was required to reveal the presence of HIV. After 90 days of treatment, no infectious virus was detectable in the PBMC of seven subjects. The viral load of the other five patients showed a substantial decrease in viral titer of between 1.28 log and 1.83 log, with an average decrease of 1.63 log. Using PCR, plasma HIV RNA values before treatment ranged from 3521 to 128973 copies/ml of plasma. After 90 days of treatment, no HIV RNA was detectable in six patients. The other six patients showed a substantial decrease in RNA copy numbers of between 1.36 log and 2.92 log, with an average decline of 1.87 log.

The reduction in viral load was accompanied by a substantial average increase in CD4 count, rising from 392 at day 0 (average percentage of CD4 relative to total lymphocytes 22.6) to 573 at day 90 (relative percentage 30.1). For the subgroup of six patients whose plasma viral load became undetectable after 90 days' treatment, the average increase in CD4 count was 283.

These results show that the combination treatment is well tolerated at the doses used, and induces a large reduction of viral load accompanied by a strong rise in CD4 count in our population. The reduction of viral load to non-detectable levels in half our population is clearly exciting. If prolonged treatement of such patients were to result at follow-up in confirmation of non-quantifiable levels, then quantification of viral load in the lymphoid tissue would be justified.

HU used in women of child-bearing age poses concerns, since antimetabolites are notorious teratogens, and it should not be used in this group. HU may also have carcinogenic potential Donehower, 1992. It will therefore be important to develop other similar compounds with less risk of these kinds of toxicity.

A randomised multicentre European trial is scheduled to begin within the next few months.

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