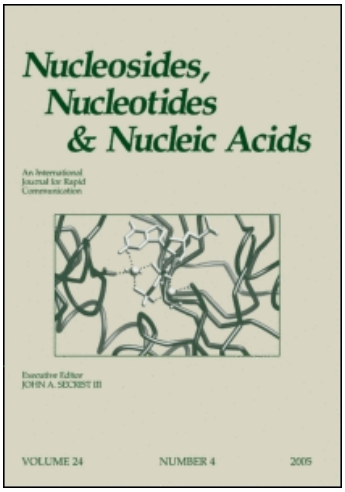

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INHIBITION OF HIV-1 REPLICATION IN MACROPHAGES BY RED BLOOD CELL-MEDIATED DELIVERY OF A HETERODINUCLEOTIDE OF LAMIVUDINE AND TENOFOVIR

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□ *Homo- and heterodimers of nucleoside/nucleotide analogues as reverse transcriptase inhibitors are effective on HIV-1-infected human monocyte-derived macrophages (M/M) compared to the single drugs or their combination. Since the combined treatment of lamivudine (3TC) and tenofovir ((R)PMPA) has an antiretroviral efficacy and a synergic effect respect to separate drugs, the heterodinucleotide 3TCpPMPA was synthesized. A single administration of the dimer as free drug or 3TCpPMPA-loaded RBC selectively targeted to M/M was able to almost completely protect macrophages from "de novo" infection.*

Keywords Heterodinucleotides; anti-HIV-1 activity; human macrophages

INTRODUCTION

The combination of nucleoside analogues as reverse transcriptase inhibitors (NRTIs) with nucleotide analogues or other antiviral drugs resulted in effective therapy for the treatment of human viral infections. Despite the clinical benefit associated with the highly active antiretroviral therapy (HAART), current drugs are not able to eradicate HIV-1 simply due to the persistence of the virus in cellular reservoirs, predominantly long-lived memory CD4+ T cells and cells of the macrophage lineage.[1] Indeed, HIV-1 has been recovered from the peripheral blood monocytes of patients with maximal viral suppression while on HAART.[2] Viral reservoirs established

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early during the infection remain unaffected by antiretroviral therapy for a long time and are able to replenish systemic infection following the failure or cessation of the treatment.

Since the crucial role of the monocyte-derived macrophages (M/M) as a HIV-1 reservoir, therapeutic strategy must consider the inhibition of the viral replication in M/M.[3]

However, since macrophages are resting cells, they phosphorylate (activate) the antiviral nucleoside analogues at a rate lower than that of peripheral blood mononuclear cells.[4] Thus, the delivery of phosphorylated nucleoside analogues directly into infected-macrophages could result in highly anti-HIV-1 activity.

On this basis, some antiviral double-drugs (homo- and heterodinucleotides) as reverse transcriptase inhibitors and as potential pro-drugs with efficacy against HIV-1-infected M/M have been reported by us.[5] Herein, we report on the synthesis of a new heterodinucleotide 3TCpPMPA consisting of the potent anti-HIV-1 nucleoside and nucleotide analogues lamivudine (3TC) and tenofovir ((*R*)PMPA), respectively.

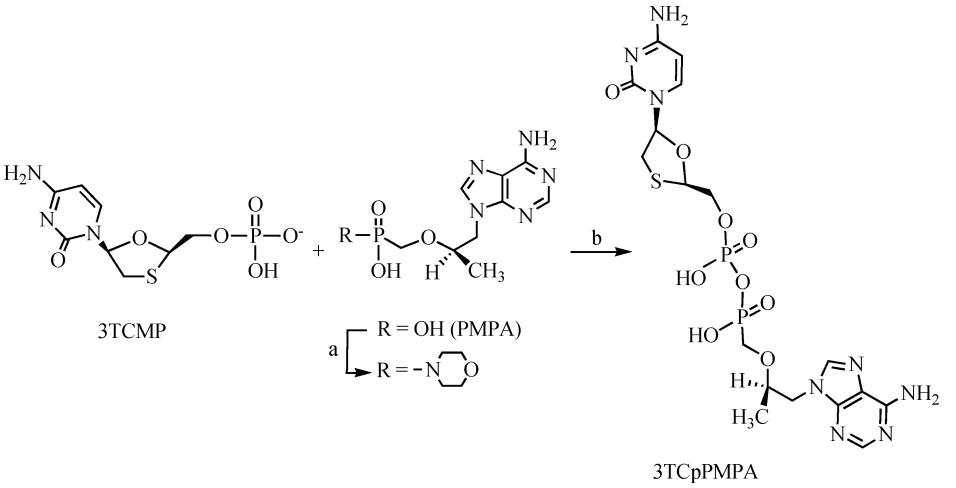
The combination of tenofovir and lamivudine has already been suggested in therapy guidelines for HAART-naïve patients.[6] The efficacy of the dimer 3TCpPMPA to protect the refractory cell compartment against de novo HIV infection by simultaneous administration of 3TC and (*R*)PMPA in a single molecule has been assessed.

RESULTS AND DISCUSSION

The heterodinucleotide consisting of 3TC and (*R*)PMPA bound together by a phosphate bridge was synthesized by coupling the mono *n*-tri-butylammonium salt of β-L-3′-thia-2′,3′-dideoxy-cytidine monophosphate (3TCMP) with (*R*)-9-(2-phosphonometoxypropyl)adenine as a morpholidate derivative (Scheme 1).

At first, the metabolism of 3TCpPMPA in plasma obtained from healthy seronegative donors was determined. As shown in Figure 1, human plasma possesses enzymes able to cleave the phosphate bridge of 3TCpPMPA with the stoichiometric production of PMPA and 3TC (the latter is quickly obtained starting from 3TCMP). The results obtained show that 3TCpPMPA is stable enough in human plasma ($t_{1/2} = 4$ hours) and suggest the possibility of using 3TCpPMPA as a prodrug of 3TC and PMPA.

The ability of the heterodimer to protect the infected human macrophages against de novo HIV-1 infection also was determined. Compound was administered to macrophages and maintained all through experiment at a concentration of 1.0 μM and virus production was evaluated over a period of 35 days. The results show an almost 100% inhibition of viral replication (Table 1).



SCHEME 1 Reagents and conditions: a) morpholine, dicyclohexylcarbodiimide, *t*-BuOH/H_2O, reflux; b) N,N-dimethylformamide, 40°C.

The selective delivery of 3TCpPMPA to macrophages was performed using a drug targeting system. This system is based on the use of autologous artificially-aged red blood cells (RBC)[7] that allows the direct administration of nucleoside analogues, already in phosphorylated form, to macrophages. The heterodinucleotide was encapsulated into autologous erythrocytes by

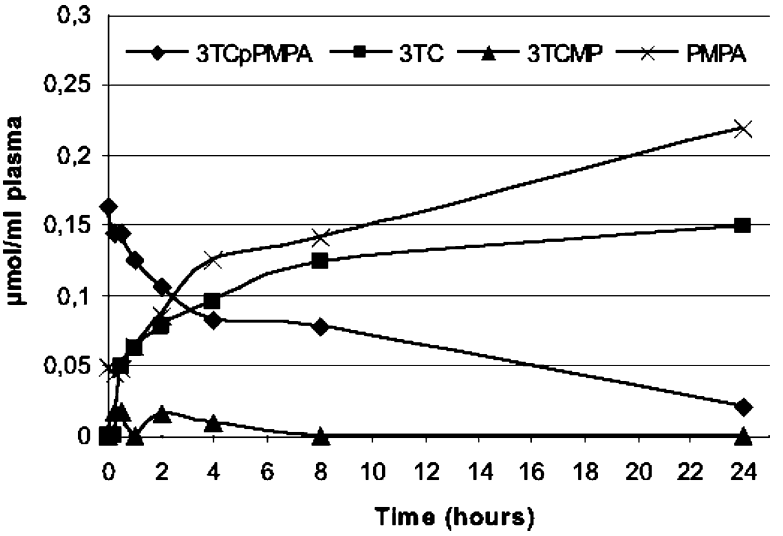


FIGURE 1 Metabolism of 3TCpPMPA in human plasma. 3TCpPMPA (0.25 mM) was incubated in human plasma for 24 hours at 37°C. Perchloric acid extracts were prepared at different incubation times and analyzed by HPLC, as described above. All values are the mean (± S.D.) of three different experiments.

TABLE 1 Percentage of virus production in HIV-1-infected human macrophages

Virus production (% of infected control)	
Time (days)	3TCpPMPA (1.0 μM)
14	0
21	0
28	0.3
35	0.5

Values are expressed in percentage of p24 production respect to untreated infected macrophages. All values are the mean (± S.D.) of sextuplicate cultures of three representative experiments and show the percentage of inhibiton in p24 produced until 35 days after infection. One hundred percent virus production corresponds to 23625 ± 2902 pg p24 antigen/mL.

a procedure of hypotonic dialysis, isotonic resealing, and reannealing.[7] 3TCpPMPA-loaded erythrocytes were modified to increase their phagocytosis by human macrophages. Then, the cells were infected by HIV-1_{Ba-L} and inhibition of HIV-1 replication was assessed by HIV $p24^{gag}$ quantification. When 3TCpPMPA was selectively targeted to the macrophagic compartment by a single administration of loaded erythrocytes, the protection of macrophages from de novo infection was nearly complete (99% protection 3 weeks posttreatment).

In conclusion, the heterodinucleotide 3TCpPMPA could act as an efficient prodrug that, by the conversion in 3TCMP and PMPA inside macrophages, is able to protect the refractory cell compartment against de novo HIV-1 infection for a long time.

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