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Short communication

Potent antiviral activity of amprenavir in primary macrophages infected by human immunodeficiency virus

Stefano Aquaro [a,*], Tania Guenci [a], Fabiola Di Santo [a], Mauro Francesconi [b], Raffaele Caliò [a], Carlo Federico Perno [a]

[a] *Department of Experimental Medicine and Biochemical Sciences, University of Rome Tor Vergata, Via Montpellie 1, Rome 00133, Italy*
[b] *Haematology Center, Frascati Hospital, Frascati, Italy*

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Abstract

Objective of the present study was then to assess the antiviral activity of the protease inhibitor amprenavir in macrophages (M/M), and to compare it with its efficacy in peripheral blood lymphocytes (PBL). M/M were obtained from blood of sero-negative healthy donors and infected with M-tropic HIV-1 strain (HIV-$1_{Ba\text{-}L}$). The stabilized infection was assessed by monitoring the HIV-1 p24 gag antigen production in the supernatants of M/M cultures. In the setting of acute infection (treatment before HIV-1 challenge), amprenavir showed substantial activity both in M/M and PBL at similar concentrations (EC_{50}: 0.011 and 0.031 μM, respectively); complete inhibition of HIV-1 replication was achieved in both cell types at concentration of about 2 μM. In the setting of chronical infection (i.e. antiviral treatment several days after established infection), an antiviral effect of amprenavir was achieved in M/M, but at concentrations higher than those active in acutely infected M/M (EC_{50}: 0.72 μM, EC_{90}: 18.2 μM). The antiviral effect in chronically infected M/M was sustained for at least 2 weeks of continuous treatment. These findings suggest that amprenavir (at relatively high concentrations) has a clinically relevant antiviral effect in persistently infected reservoirs of HIV.

Keywords: Protease inhibitors; Therapy; Reservoirs; HAART; CD4-lymphocytes

Protease inhibitors are antiviral drugs acting at stages of virus replication occurring later than integration and transcription (McQuade et al., 1990; Meek et al., 1990; Roberts et al., 1990). Even if they are unable to block virus infection and integration of proviral genome into cellular DNA, their strong interference with the maturation and assembly of newly produced virus particles heavily affects the ability of virus infection to propagate to uninfected cells (Perno et al., 1998; Aquaro et al., 2002).

The peculiar antiviral mechanism of action of protease inhibitors (PIs) suggests their potential ability to target virus replication in cells characterized by a long-term productive chronical infection without evidence of HIV-1-mediated cytopathic effects (persistently infected cells). Such persistently infected cells are represented in the body mainly by cells of macrophage lineage (M/M), either in the systemic compartment or in the central nervous system (CNS), where they account for >90% of cells infected by HIV-1 (Orenstein et al., 1997; Meltzer et al., 1990; Koenig et al., 1986; Lipton and Gendelman, 1995; Tyor et al., 1993). Beyond direct infection, M/M are able to transfer the virus to $CD4^+$ T lymphocytes, and trigger apoptosis of bystander T lymphocytes and neural cells (Crowe et al., 1990; Mastino et al., 1993; Badley et al., 1997; Herbein et al., 1998; Aquaro et al., 2000a; Mollace et al., 2002). Therefore, it is conceivable that productively HIV-1 infected M/M represent an important source of HIV-1 and a cellular reservoir that challenges the attempts to eradicate the virus from HAART-treated patients. In these conditions of chronical infection, RT inhibitors are totally ineffective, while PIs may provide a substantial effect of potential clinical relevance (Aquaro et al., 1997a,b). Aim of this study was to evaluate the antiviral effect of amprenavir in acutely and chronically HIV-infected M/M.

Primary M/M were purified as previously described (Aquaro et al., 2000b; Perno and Yarchoan, 1993), treated with various concentrations of amprenavir, and infected with 300 $TCID_{50}$/ml of a monocytotropic isolate of HIV-1,

* Corresponding author. Tel.: +39-06-72596553; fax: +39-06-72596039.
E-mail address: aquaro@uniroma2.it (S. Aquaro).

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HIV-1_{Ba-L}. As shown in Fig. 1A, antiviral activity of amprenavir in acutely infected M/M (i.e. treated with drugs prior to virus challenge) was dose-dependent, from >99% inhibition at 4 μM, down to about 28.7% inhibition with 0.0064 μM. As a control, and consistent with previously published data, 0.1 μM AZT induced about 90% inhibition of virus replication in these acutely infected M/M (Fig. 1A). EC_{50} and EC_{90} of AZT were 0.011 and 0.032 μM, respectively (Table 1). To study the activity of amprenavir in chronically infected M/M, antiviral treatment was started at 10 days after infection (day 0), that is at the time of stable virus production. Release of p24-mature gag antigen (Ag) by chronically infected M/M was markedly decreased (compared to control) by day 2 after drug treatment with the highest concentrations of amprenavir (4 and 20 μM) (Fig. 1B), and became even more pronounced afterwards; starting from this time point, and until the end of the experiment, a substantial and stable (up to day 12 after treatment) inhibition of the release of HIV-1 p24 gag Ag was achieved with concentrations of amprenavir of 4 and 20 μM (73.2 and 91% at day 12, respectively). EC_{50} and EC_{90} of amprenavir in chronically infected M/M were 0.72 and 18.2 μM, respectively, that is 70- to 600-fold higher than the EC_{50} and EC_{90} of amprenavir in acutely infected M/M (Table 1). Interestingly, no complete inhibition of virus replication could be achieved even with the highest non-toxic concentrations tested (Fig. 1B, and Table 1). AZT 20 μM (a concentration about 500-fold higher than its EC_{50} in M/M acutely infected by HIV-1), was completely ineffective against the production of HIV-1 p24 gag Ag in chronically infected M/M (Fig. 1B), further confirming the absence of new rounds of replication in these cells after day 10 of infection (day 0) (Perno et al., 1993, 1994, 1998; Aquaro et al., 2002).

Peripheral blood lymphocytes (PBL) were plated in 48-well plates in the presence or absence of various concentrations of drugs, and challenged 30 min later with 300 $CCID_{50}$/ml of HIV-1_{IIIB}. Antiviral activity of amprenavir in acutely infected PBL was similar to that reported previously by other authors (St. Clair et al., 1996), with an EC_{50} and EC_{90} of 0.031 and 0.67 μM, respectively, in the range of those recorded for acutely infected M/M (Table 1). Such effect was maintained up to the end of the experiments (Fig. 1C). EC_{50} and EC_{90} of amprenavir in PBL were 23- and 298-fold lower than the EC_{50} and EC_{90} in chronically infected M/M ($P < 0.001$ for each comparison) (Table 1). Treatment of M/M and PBL with concentrations of amprenavir up to 20 μM showed no decrease in cell number, thus suggesting the absence of major toxicity. To assess the potential effect of DMSO upon infected cells, appropriate controls were run by using concentrations of DMSO corresponding to those present in both drug-treated M/M and PBL (Fig. 1).

We report in this paper that HIV-1 production is inhibited in vitro by amprenavir in chronically infected M/M at clinically relevant concentrations. However, the activity of amprenavir in chronically infected M/M is several fold lower than that in acutely infected M/M, without achieving complete inhibition of HIV-1 p24 production in chronically infected M/M even at the highest concentrations of drugs tested. This latter finding agrees with our and other previously published results using other protease inhibitors in M/M exposed to HIV-1, thus showing that relatively high concentrations of amprenavir are required to suppress HIV-1 production in chronically infected M/M. From a practical viewpoint, two conclusions can be drawn: (a) amprenavir achieves a remarkable antiviral effect in chronically infected M/M at concentrations in the range or even lower than the through level ($C_{through}$) achieved in patients treated with amprenavir, particularly if boosted by ritonavir (Sadler et al., 1999) (the association most commonly used). This confirms that amprenavir may play a relevant role in inhibiting virus replication even in chronically infected M/M, thus interfering with HIV also in persistently infected reservoirs; (b) at the same time, the minimal virus replication even with the highest concentration used, and the block at post-integrational events in the HIV lifecycle, suggests that such cells may escape complete HIV-1 suppression in patients receiving these drugs (Aquaro et al., 2002). This underscores the possibility that virus production may rebound immediately after drug withdrawal from chronically

Table 1
Anti-HIV activity of amprenavir in macrophages and T lymphocytes

Cells	EC_{50} (μM)	EC_{90} (μM)	CC_{50} (μM)
Amprenavir			
M/M			
Acutely infected	0.011 (±0.003)	0.032 (±0.002)	>20
Chronically infected	0.72 (±0.05)	18.2 (±0.5)	>20
PBL			
Acutely infected	0.031 (±0.003)	0.67 (±0.05)	15
AZT			
M/M			
Acutely infected	0.01 (±0.004)	0.08 (±0.002)	>100
Chronically infected	Not effective	Not effective	>100
PBL			
Acutely infected	0.2 (±0.04)	1.4 (±0.2)	20

The geometric mean of p24 gag Ag production of replicates in each experiment was used to determine the effective drug concentration where 50 and 90% of viral replication is inhibited (EC_{50} and EC_{90}, respectively), by linear regression of the log of the percent HIV-1 p24 production (compared to untreated controls) vs. the log of the drug concentration. The EC_{50}s and the EC_{90}s for acutely infected PBL and chronically infected M/M were calculated using supernatants collected at the end of experiment, that is 12 days after treatment of drug; for acutely infected M/M EC_{50} and EC_{90} values were calculated 24 days after infection (superimposable to those obtained at day 12, see results). Macrophages and PBL were treated for 7–14 days in the presence of different concentrations of amprenavir or AZT. Cytotoxic concentration 50% (CC_{50}) was visually assessed (and compared to untreated controls) using the trypan blue exclusion method. In the case of M/M, cells were previously gently detached from the wells as described elsewhere (Perno et al., 1993). Results represent the average of one out of two experiments each run in triplicate.

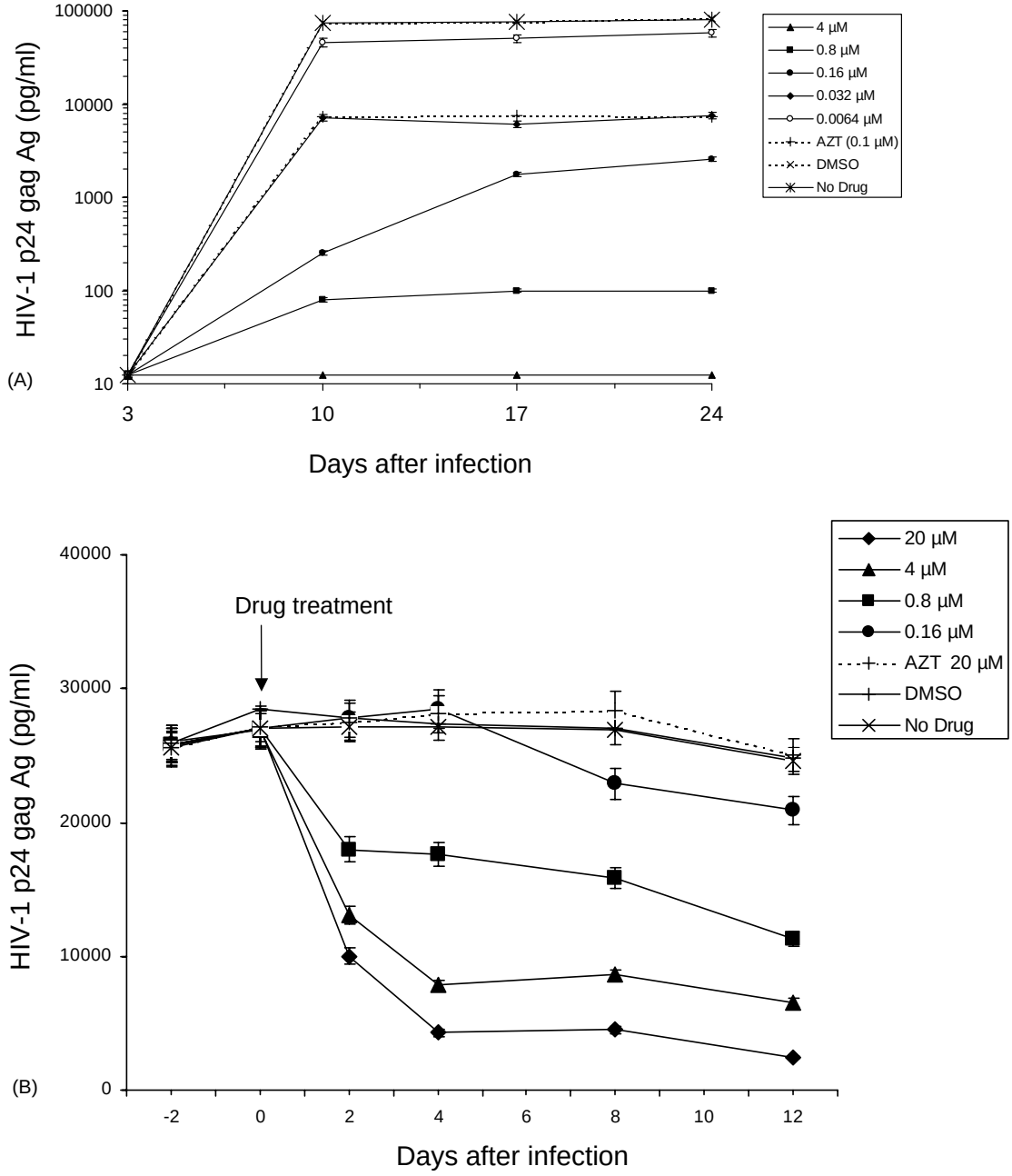
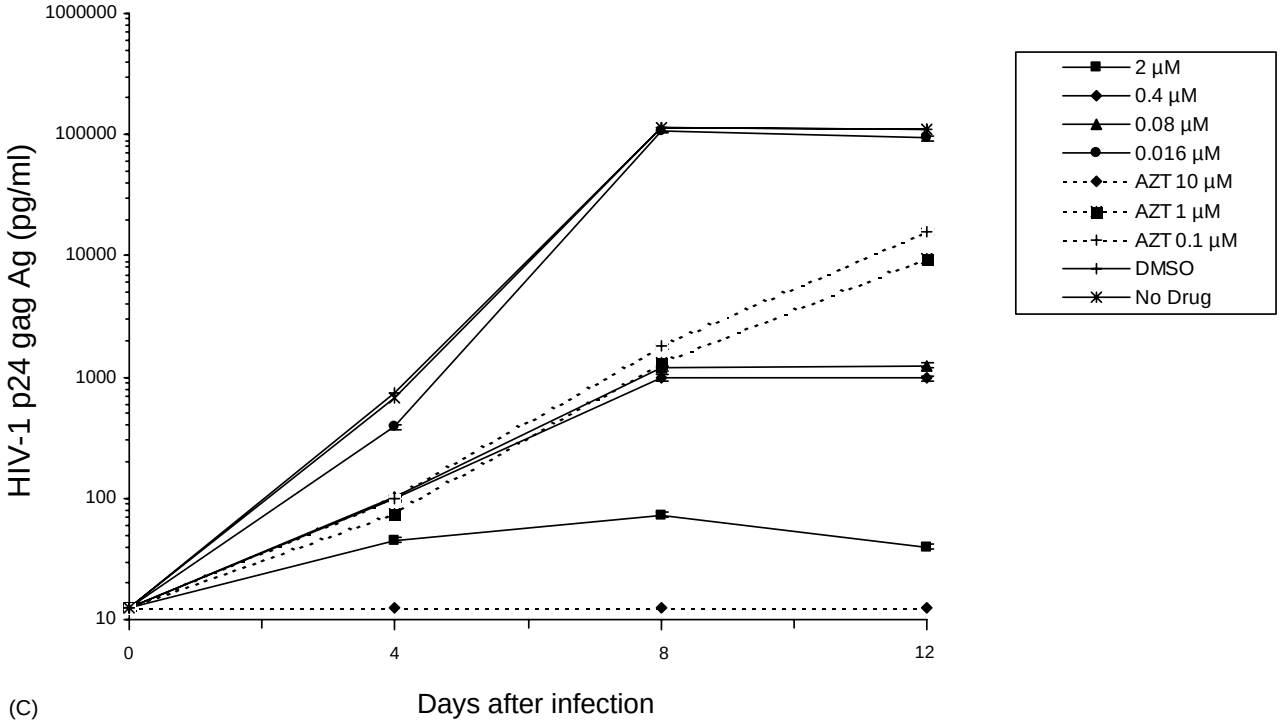


Fig. 1. (A) Anti-HIV-1 activity of amprenavir in acutely infected M/M. Peripheral blood mononucleated cells (PBMC) obtained from healthy HIV-1-negative donors were separated over Ficoll gradient and seeded in 48-well plates at 1.8×10^6 cells/well in 1 ml of RPMI 1640 containing 20% heat-inactivated, endotoxin and mycoplasma-free fetal bovine serum (Hyclone Laboratories, Inc., Logan, UT), 4 mM L-glutamine (Life Technologies), 50 U/ml penicillin and 50 μg/ml streptomycin (Life Technologies) (complete medium). Five days after plating and culturing the PBMC at 37 °C in a humidified atmosphere enriched with 5% CO_2, non-adherent cells were carefully removed by repeated washings with warmed RPMI 1640, leaving a monolayer of adherent cells which were finally incubated in complete medium. Drugs were added to M/M 30 min before virus challenge, and kept in culture throughout the experiments. Stock solutions from pure amprenavir and AZT powder used in these experiments were made in dimethyl sulfoxide (DMSO), antiviral activity of amprenavir at concentrations above 20 μM could not be assessed due to DMSO cytotoxic effect. Results represent the average of a representative experiment out of two, each run in triplicate. (B) Kinetics of HIV-1 p24 gag Ag production in supernatants of chronically infected M/M treated with amprenavir. On day 10 after infection (first day of treatment), the drugs were added to chronically infected M/M. Data are the average from a single experiment (each sample run in quadruplicate) which is representative of three different experiments. (C) Anti-HIV-1 activity of amprenavir in acutely infected PBL. PBL were purified from PBMC by repeated adherences to remove monocytes, and then cultured with the same medium as M/M, supplemented with 2 μg/ml phytohemagglutinin (PHA). Stimulation was carried out for 72 h; afterward, the medium was discarded, cells were washed three times with RPMI 1640 and the concentration was adjusted to 5×10^5 cells/ml of medium supplemented with 50 U/ml recombinant interleukin-2 (IL-2). Drugs were added to M/M at 30 min before challenge with 300 $CCID_{50}$/ml of HIV-1_{IIIB}. Results represent the average of one out of two experiments each run in triplicate. Virus production was determined by measuring HIV-1 p24 gag Ag released in the supernatants of cell cultures using a commercially available kit (Abbott labs, Pomezia, Italy). The error bars show S.D.



(C)

Fig. 1. (*Continued*).

infected cells with integrated HIV DNA. This phenomenon may somewhat contribute to the rapid reappearance of virus in plasma of patients whose therapy has been interrupted for any reason (Hatano et al., 2000). The partial effect of HIV inhibitors in chronically infected M/M may also help in explaining the discordant results found with HAART for the plasma and the CNS, where chronically infected M/M are the major source of HIV-1, and may also contribute to the discordant rate of mutations conferring resistance to antiretroviral drugs in these two compartments.

In our experimental model, by days 10–12 after virus challenge, about 50% of M/M in the cultures are infected by HIV-1, and this number remains stable for a long period of time (Aquaro et al., 2002). It is worth stressing that AZT, used in these experiments in chronically infected M/M, was not able to inhibit HIV-1 replication; indeed, protease inhibitors (such as amprenavir) are the only drugs effective in these cells, because protease inhibitors (but not RT inhibitors) are active at post-integrational steps of HIV-1 replication, when new rounds of reverse transcription are no longer occurring.

We cannot exclude in principle that the different activity of amprenavir between acutely infected lymphocytes and chronically infected M/M could be related to the different virus strains used in the two experimental models (IIIB T-tropic and Ba-L M-tropic, respectively). However, it was previously shown that reverse transcriptase (RT) inhibitors are equally active in macrophages infected with either lymphocytotropic or monocytotropic isolates of HIV-1 (Crowe et al., 1989; Perno et al., 1988). In addition, the amino acid sequences of both the RT and the protease of HIV-$1_{Ba\text{-}L}$ are nearly identical to that of the corresponding proteins of all wild-type lymphocytotropic isolates studied (Cenci et al., 1997). This strongly suggests that the difference in term of antiviral activity of amprenavir between acutely infected lymphocytes and chronically infected M/M is due to the intrinsical properties of virus replication in these cells (Aquaro et al., 2002). In conclusion, data support the utilization of protease inhibitors, such as amprenavir, for the inhibition of virus replication in the compartment of long-living persistently infected cells (mainly M/M). This also supports a differential effect of HAART regimens containing PIs or not containing PIs, where the former may affect the compartment of persistently infected cells, while the latter are more specific for the inhibition of new rounds of infection (while they are not effective in chronically infected cells). Our results support the use of regimens containing protease inhibitor(s) with reverse transcriptase inhibitors particularly in late stages of the disease, when both clinical data and experimental models (Orenstein et al., 1997; Igarashi et al., 2001) show the key role of infected M/M in sustaining the production of new viral particles not only in the brain, but also in the systemic compartment.

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