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Mini-review

Therapeutic strategies towards HIV-1 infection in macrophages

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Dedicated to Prof. Erik De Clercq on the occasion of reaching the status of Emeritus-Professor at the Katholieke Universiteit, Leuven in September 2006.

Abstract

It is widely recognized that macrophages (M/M) represent a crucial target of HIV-1 in the body and play a pivotal role in the pathogenic progression of HIV-1 infection. This strongly supports the clinical relevance of therapeutic strategies able to interfere with HIV-1 replication in M/M. In vitro studies showed that nucleoside analogue inhibitors of HIV-1 reverse transcriptase have potent antiviral activity in M/M, although the limited penetration of these compounds in sequestered body compartments and low phosphorylation ability of M/M, suggest that a phosphonate group linked to NRTIs may confer greater anti-HIV-1 activity in M/M. Differently, the antiviral activity of non-nucleoside reverse transcriptase inhibitors in M/M is similar to that found in CD4+ lymphocytes. Interestingly, protease inhibitors, acting at a post-integrational stage of HIV-1 life-cycle are the only drugs active in chronically infected M/M. A careful analysis of the distribution of antiviral drugs, and the assessment of their activity in M/M, represent key factors in the development of therapeutic strategies aimed to the treatment of HIV-1-infected patients. Moreover, testing new and promising antiviral compounds in such cells may provide crucial hints about their efficacy in patients infected by HIV.

Keywords: HIV-1; Macrophages; CD4+ lymphocytes; Antiretroviral drugs

Contents

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1. Introduction

Macrophages (M/M) are widely recognized as the second cellular target of HIV-1, and a crucial virus reservoir (Gartner et al., 1986; Lane et al., 1996; Herbein et al., 2002). HIV-1-infected M/M are widely distributed in all tissues and organs (Koenig et al., 1986; Tschachler et al., 1987; McElrath et al., 1989), including the central nervous system (CNS) where they represent the majority of cells infected by HIV-1 (Gabuzda et al., 1986; Tyor et al., 1993).

HIV-1 replication in M/M is a crucial pathogenic event during the progression of HIV-1 infection. In fact, productively infected M/M can fuse with CD4+ lymphocytes and transfer the virus to these cells in the context of the antigen presentation and the immune response (Crowe et al., 1990); in addition infected M/M release cytotoxic factors that can mediate the activation of programmed cell death on bystander cells such as CD4+ and CD8+ lymphocytes (Badley et al., 1997; Herbein et al., 1998; Garaci et al., 2003), neurons and astrocytes even without a direct infection of these cells (Shi et al., 1996; Aquaro et al., 2000a; Mollace et al., 2002). In agreement with this result, as few as 500 HIV-1-infected M/M have been demonstrated to be able to completely deplete millions of autologous CD4+ lymphocytes in a SCID mouse model (Garaci et al., 2003).

HIV-1 infection in M/M is characterized by viral dynamics substantially different from that of CD4+ lymphocytes. In fact, activated CD4+ lymphocytes can sustain a rapid and exponential viral production followed by massive cell death (Bagnarelli et al., 1996). In contrast, M/M are resistant to the cytopathic effect of HIV-1 (Gendelman et al., 1988; Orenstein et al., 1988) and produce virus over a prolonged period, with dynamics that increases linearly during the first 1–2 weeks of infection, followed by a plateau of high level of replication (>10^8 copies of unspliced/spliced RNA produced) lasting at least up to 60 days after infection (Aquaro et al., 2002).

M/M can survive HIV-1 infection for long periods of time. This is mainly related to autocrine secretion of the nerve growth factor (NGF) associated with enhanced expression of the high-affinity NGF receptor p140 trkA on their surface. This complex interaction enhances the ability of macrophages to cope with HIV infection, thus transforming them in a powerful, long-term infected, viral reservoir (Garaci et al., 1999).

The dynamics of virus replication, quite different in M/M and CD4+ lymphocytes, may suggest that anti-HIV-1 drugs act differently in these cells. On this basis, we reviewed the characteristics of the activity in M/M of antiviral compounds of clinical interest, as well as the factors affecting their efficacy. In addition, new and innovative anti-HIV-1 therapeutic approaches directed to HIV-1-infected M/M are briefly described.

2. Reverse transcriptase inhibitors

To date, 11 out of 21 compounds approved for the treatment of HIV-1 infection are reverse transcriptase inhibitors (RTIs). In particular, they consist of the group of 7 nucleoside-analogue RTIs (NRTIs: ZDV-zidovudine, d4T-stavudine, 3 TC-lamivudine, ddI-didanosine, ABC-abacavir, ddC-zalcitabine, FTC-emtricitabine), 1 acyclic nucleoside phosphonate (TDF-tenofovir), and 3 non-nucleoside analogue RTIs (NNRTIs: NVP-nevirapine, DLV-delavirdine, and EFV-efavirenz).

Table 1
2′-Deoxynucleoside-triphosphate levels in resting macrophages and lymphocytes

dNTP	M/M (pmoles/10^6cells)[a]	PBL (pmoles/10^6cells)[b]	Ratio
dATP	5.13	99.11	19.3
dCTP	14.21	88.45	6.2
dGTP	8.65	127.30	14.7
dUTP	4.34	106.40	24.5
dTTP	19.15	451.55	23.6

Table reports data from Aquaro et al. (1998).
[a] Macrophages: human primary M/M obtained from the blood of healthy seronegative donors.
[b] Peripheral blood lymphocytes.

2.1. Nucleoside-analogue reverse transcriptase inhibitors

The activity of NRTIs depends on two factors: (1) the intracellular concentration of their triphosphorylated moiety since NRTIs require triphosphorylation by cellular kinases to act as competitors of the natural 2′-deoxy-nucleoside trisphosphates (dNTPs); (2) the concentration of the cellular dNTP pools. M/M are resting cells, characterized by a limited DNA synthesis and consequently by low intracellular level of dNTPs (Perno et al., 1988). The resting status of M/M overcomes the low affinity of most NRTIs for kinases acting at their first phosphorylation step (Ahluwalia et al., 1987; Balzarini et al., 1987, 1988, 1989; Johnson and Fridland, 1989; Hao et al., 1990), and results in a competition by dNTPs lower in M/M than in CD4+ lymphocytes whose intracellular dNTPs pool is 6–20-fold greater than those found in M/M (Table 1) (Aquaro et al., 1997, 1998). These metabolic characteristics may explain why all the NRTIs clinically available are more active in M/M than in CD4+ lymphocytes in in vitro biological models (Table 2).

2.1.1. Acyclic nucleoside phosphonates

In sequestered body compartments, such as the CNS, the intracellular concentrations of NRTI triphosphates may be suboptimal (Table 2) as a consequence of their low penetration in these compartments (Lewis et al., 1996; Haworth et al., 1998) coupled with the high expression of p170 glycoprotein in M/M able to excrete NRTIs across the cellular membrane (Malorni et al., 1998). In order to circumvent the bottleneck represented by the first phosphorylation step, acyclic nucleoside phosphonates, in which a phosphonate group is linked to the alkyl side chain of purines or pyrimidines, have been synthesized. Tenofovir disoproxil fumarate (TDF), the first acyclic nucleoside phosphonate approved for clinical use, has a potent anti-HIV-1 activity in M/M (Table 2). This remarkable efficacy may be explained mainly by two main reasons: (1) the bypass by tenofovir of the first phosphorylation step (notoriously slow in M/M) since this compound is already equipped by a phosphonate group linked to the alkyl side chain of the adenine (De Clercq et al., 1986,

Table 2
Activity of anti-HIV drugs in acutely infected T-lymphocytes (PBL) and macrophages (M/M) and in chronically infected macrophages (M/M)

Drugs	EC_{50}[a] (acute infection) (μM)[b]		EC_{50}[a] (chronic infection) (μM)[c]	C_{max}[d] range (μM)	
	PBL	M/M	M/M	Plasma	Liquor
Nucleoside RT inhibitors					
Zidovudine	0.2	0.02	n.e.	4.49–6.64	0.12–0.41
Didanosine	0.5	0.05	n.e.	2.12–11.0	0.17–0.51
Zalcitabine	0.04	0.003	n.e.	0.05–0.18	0.003–0.03
Lamivudine	0.04	0.02	n.e.	4.37–8.74	0.05–1.14
Stavudine	0.8	0.24	n.e.	3.35–6.43	0.2–0.36
Abacavir	0.9	0.3	n.e.	5.2–10.9	0.5–1.83
Tenofovir	0.37	0.02	n.e.	0.35–0.38	–
Non-nucleoside RT inhibitors					
Nevirapine	0.04	0.05	n.e.	7.52–16.92	1.3–10.9
Delavirdine	0.006	0.01	n.e.	15–55	0.02–0.22
Efavirenz	0.01	0.01	n.e.	9.2–16.6	0.006–0.09
Protease inhibitors					
Saquinavir	0.01	0.05	0.5	1.85–3.23	0.003–0.008
Indinavir	0.05	0.06	0.4	12.2–13	0.03–0.66
Ritonavir	0.02	0.12	3.3	10.5–26	0.003–0.032
Nelfinavir	0.04	0.08	1.4	5.63–8.45	0.003–0.012
Amprenavir	0.03	0.01	0.72	10.6–19.2	0.003–0.36
Fusion inhibitor					
T-20	0.01	0.02	n.d.	0.39–1.11	<0.005[e]

Data from Perno et al. (1994, 1998), Aquaro et al. (1997), Kilby et al. (2002), Ketas et al. (2003), Price et al. (2005); n.e.: not effective; n.d.: not defined.
[a] Effective concentration 50%.
[b] Acutely infected PBL and M/M: antiviral treatment started before virus challenge, before HIV–DNA integration.
[c] Chronically infected M/M: antiviral treatment started after virus challenge, when HIV–DNA is already integrated within the cellular genome.
[d] The maximal concentration of drugs.
[e] This value is below the assay detection limit.

1987); (2) the low dATPs levels (competing with tenofovir) in M/M (Table 1). As a consequence, the ratio between diphosphorilated tenofovir and dATP is at least 100-fold greater in M/M than in CD4+ lymphocytes, thus explaining the high activity of tenofovir in M/M (Balzarini et al., 1996a; Naesens et al., 1998). It is conceivable that the antiviral effect of tenofovir in M/M plays a role in its excellent clinical efficacy.

Overall findings confirm that DNA chain termination represents the major mechanism involved in the antiviral effect of NRTIs in M/M.

2.2. Non-nucleoside reverse transcriptase inhibitors

Differently from the NRTIs, the NNRTIs are non-competitive inhibitors of HIV-1 reverse transcriptase that bind at a hydrophobic pocket near the polymerase active site. These compounds do not prevent the binding of nucleoside triphosphate substrates to reverse transcriptase, but block the polymerization reaction and thus the ability of reverse transcriptase to synthesize viral DNA. Since the NNRTI activity is not affected by the dNTP pools, substantial differences in antiviral activity of NNRTI have not been observed between M/M and CD4+ lymphocytes (Table 2). As a confirmation of these findings, the anti-HIV-1 activity of NNRTIs is not modulated by the macrophage colony stimulating factor, which increases the dNTPs pool in M/M and thus affect the activity of NRTIs (Aquaro et al., 1997).

3. Protease inhibitors (PIs)

Protease inhibitors, acting at a post-integrational step of HIV-1 life-cycle, are the only drugs able to suppress HIV-1 replication in chronically infected M/M characterized by the proviral DNA already integrated into the genome. In particular, all PIs clinically available showed a remarkable antiviral activity in chronically infected M/M, even if their activity occurs at concentrations greater than those required in CD4+ lymphocytes (Table 2) (Perno et al., 1993, 1994, 1998; Aquaro et al., 2004). The lower activity of PIs in M/M versus CD4+ lymphocytes (opposite of that described for NRTIs in acutely infected M/M and lymphocytes) may be due to the high and sustained RNA metabolism in M/M that affords a great production of virus particles even from a limited amount of proviral DNA in these cells. As a confirmation of this hypothesis, our findings demonstrated that in HIV-1-infected M/M the intracellular HIV-RNA production increases during the first 1–2 weeks of infection followed by a plateau, and PIs do not affect this production (Aquaro et al., 2002).

These findings may have clinical consequences. First of all, the high concentration of PIs required to suppress HIV-1 replication in chronically infected M/M is often at the upper limit, or even beyond, the through PI concentration achievable in plasma of treated patients. Thus, tissue M/M chronically infected by HIV-1 may escape HIV-1 suppression, particularly in patients

with a poor compliance to therapy or an altered drug absorption or metabolism. The only exception is represented by amprenavir. Indeed, although high concentration of amprenavir is required to inhibit HIV-1 replication in chronically infected M/M (Aquaro et al., 2004), this drug is characterized by a favourable adsorption and clearance pharmacokinetics (especially if boosted with ritonavir) able to increase the through concentration in plasma of treated patients (Sadler et al., 1999).

Moreover, since antiviral therapy is unable to affect the proviral DNA in such cells, the production of virus particles may be rapidly resumed from chronically infected M/M in the absence of PIs (Ortiz et al., 2001; Lori and Lisziewicz, 2001).

Overall findings underline the difficulty to achieve results in chronically infected M/M, but at the same time suggest the importance of PIs as drugs able to interfere with the late stages of the HIV-1 life-cycle. For these reasons, the use of PIs at an appropriate dose and in combination with NRTIs may represent an important tool in clinical practice.

4. Fusion inhibitors

T-20 (Fuzeon/Enfuvirtide), the first fusion inhibitor approved for clinical practice, is a synthetic peptide that potently inhibits HIV-1 replication by interfering with the transition of the envelope glycoprotein gp41 (essential for mediating the fusion between the virus and the host cell membrane) to a fusion active state.

T-20 has characteristics completely different from the other currently available therapeutic approaches since the development of T-20 resistance leads to the selection of mutations able to reduce the HIV-1 cytopathic effect and/or to enhance the immune response (Aquaro et al., 2006). T-20 is broadly active in blocking the entry of HIV-1 into PBMC, M/M, and immature dendritic cells, even if cell type differences in potency were observed (Ketas et al., 2003). These differences may reflect the modulation of T-20 susceptibility by coreceptor specificity. In fact, HIV-1 uses as coreceptor the chemochine receptor CXCR4 expressed by T-lymphocytes and CCR5 expressed by M/M. It was postulated that the interaction with CCR5 minimizes the time during which the T-20-binding site is exposed, thus reducing the susceptibility of HIV-1 to T-20 and conferring an intrinsic resistance of HIV-1 to T-20 (Reeves et al., 2003; Reeves and Piefer, 2005). This hypothesis may explain why higher concentrations of T-20 are required to efficiently suppress HIV-1 replication of CCR5-using strains than that of CXCR4-using strains.

5. Towards new therapeutic strategies

5.1. Masked NRTI monophosphate derivates

In order to circumvent the dependence of NRTIs on the activation by kinases, the prodrugs of the 5′ monophosphate forms of NRTIs have been synthesized (Puech et al., 1993; Perigaud et al., 1994; Balzarini et al., 1996b, 1999, 2000). In particular, the phosphoramidate triesters are characterized by a NRTI monophosphate containing an aryl group linked to the phosphorous trough an ester bond, and a methyl ester of L-alanine linked to the phosphorous trough a phosphoramidate bond with the primary amino moiety. The phosphoramidates of those NRTIs whose limiting step is the first phosphorylation have a potent anti-HIV activity (Balzarini et al., 1996b; Aquaro et al., 2000b). In contrast, the addition of monophosphate is not a useful approach for those NRTIs, such as AZT, 3TC, and ddC, whose first phosphorylation step is not a bottleneck for eventual antiviral activity (Aquaro et al., 2000b).

5.2. Viral entry inhibitors through blockade of the viral coreceptor CCR5

The chemokine receptor CCR5 (belonging to the family of the seven-transmembrane domain proteins) is expressed by M/M and represents the most important coreceptor for M-tropic R5 HIV-1 strains to enter the cells. CCR5 plays a crucial role in transmission of HIV-1 isolates that establish initial infection, persist during the early years of infection, and predominate in brain where HIV causes neuro-AIDS (Alkhatib et al., 1996; Weissman et al., 1997; Tuttle et al., 1998; Wang et al., 1998). For all these reasons, CCR5 represents an attractive therapeutic target for design of new entry inhibitors.

The CC-chemokines CCL3L1 (LD78β), CCL5 (RANTES), CCL4 (MIP-1β), CCL3 (MIP-1α/LD78α) are natural ligands for the CC-chemokine receptor CCR5, and are inhibitors of CCR5-using HIV strains (Cocchi et al., 1995; Aquaro et al., 2001). A recent study demonstrated that different HAART regimens may modify the level of chemokine expression in M/M of HIV-1-infected patients (Wasmuth et al., 2004), thus suggesting that the modification of chemokine profile should be considered for the design of an effective HAART.

TAK-779 is the first non-peptidic molecule shown to block selectively CCR5-using HIV strain replication in M/M at low concentrations (about 10 nM) by interacting directly with CCR5 in a specific binding site within the transmembrane domain helices 1, 2, 3, and 7. TAK-779 has a high affinity for CCR5, and totally inactive against CXCR4-using strains of HIV-1 (Baba et al., 1999; Dragic et al., 2000).

The current leading CCR5 antagonists vicriviroc (SCH-D, Schering-Plough), maraviroc (UK-427,857, Pfizer), and aplaviroc (873140, GlaxoSmithKline) are characterized by an excellent potency against several laboratory CCR5-using strains (Table 3) and against a broad-spectrum of a genotypically diverse panel of CCR5-using isolates (Dorr et al., 2005; Reeves and Piefer, 2005; Strizki et al., 2005). Clinical trials have demonstrated that all these compounds have a good efficacy and

Table 3
Activity of CCR5 antagonists against HIV-1 BaL in M/M

	CCR5 antagonist		
	SCH-D	UK-427,857	GSK873140
EC_{50} (μM)	0.001	0.0005	0.03

Authors' personal observation.

tolerability in HIV-infected patients. Pharmacodynamic data also suggest that these compounds have a long plasma half-life and/or prolonged CCR5 occupancy, which may explain the delay in viral rebound observed following compound withdrawal in short-term monotherapy studies (Westby and Van der Ryst, 2005). Recently, the antiviral activity of novel small molecule inhibitors of CCR5 (derived from the UK-427,857 discovery programme) have been investigated (Willey et al., 2005). Interestingly, these inhibitors show no cross-reactivity against alternative HIV coreceptors and have good efficacy against a diverse range of R5 and R5X4 HIV-1 isolates as well as HIV-2 and SIV strains. Inhibition was also observed in cell lines as well as primary PBMCs and macrophages even if the extent of inhibition is dependent on cell type and on cell surface CCR5 concentration. These findings underline the potential of CCR5 inhibitors for clinical development.

Moreover, the CCR5 antagonists may synergize with T-20, demonstrating that targeting two steps in the entry process can have a cooperative effect. In particular, the use of CCR5 antagonists may favour the switch from CCR5-using strains to CXCR4-using strains known to be more susceptible to T-20, thus contributing to increase T-20 efficacy.

Another promising compound is Peptide T, a synthetic peptide corresponding to eight amino acids (185–192) of the gp120 V2 region, proposed to function as a viral entry inhibitor selectively targeting CCR5 (Ruff et al., 2001, 2003). Peptide T efficiently binds CCR5 thus blocking HIV-1 entry in M/M and microglia and prevents the M/M-mediating apoptosis of neuronal cells (Pollicita et al., 2006).

5.3. Plant lectins as potential anti-HIV compounds with microbicidal action

Plant-derived carbohydrate-binding lectins have been recently proposed as innovative anti-HIV compounds targeting selectively the glycans of the envelope glycoprotein gp120 (Balzarini et al., 2005; Balzarini, 2005). These agents inhibit HIV infection and also prevent HIV transmission by blocking cell–cell contact. Recent studies showed that the carbohydrate-binding lectins have a remarkable antiviral activity against a variety of HIV-1 clade isolates, laboratory HIV-1 strains, and HIV-2 and proved to be not toxic in mammalian cell models (Balzarini et al., 2005; Balzarini, 2005). These compounds were found very active against CCR5-using HIV-1 strains in M/M (Balzarini et al., 2004).

Moreover, resistance to carbohydrate-binding lectins is mediated by the selection of N-linked glycosylation site deletions in HIV-1 gp120. This represents a concept fundamentally different from all the currently available therapeutic approaches. In fact, the loss of the carbohydrate shield may determine the exposure of new epitope targets of the neutralizing antibodies, thus enhancing immune response against HIV-1. For these reasons, carbohydrate-binding lectins represent promising compounds able to compromise the viability and infectivity of HIV by a synergistic action of drug-treatment and immune surveillance (Balzarini et al., 2005; Balzarini, 2005).

6. Conclusions

The characterization of M/M as infected cells able to spread virus to bystander cells, and to interfere with the homeostasis of the immune system and of the neural compartment, strongly supports the importance of inhibiting virus replication in such cells. The dynamics of virus replication in M/M, and their intrinsic biochemical and metabolic characteristics suggest that reverse transcriptase- and protease-inhibitors are able to affect virus replication in HIV-infected M/M, yet at concentrations different from those effective in activated CD4+ lymphocytes. Generally, the relevance of M/M in the pathogenesis of HIV infection underlines the importance of testing the antiviral efficacy of new compound inhibitors of different stages of the virus life-cycle (inhibitors of entry, integrase, nuclear transport, etc.) in M/M, early in development.

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