Inhibition of HIV-1 Replication by Cyclopentenone Prostaglandins in Acutely Infected Human Cells
Evidence for a Transcriptional Block

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Abstract

Cyclopentenone prostaglandins (PGs) inhibit virus replication in several DNA and RNA virus models, in vitro and in vivo. In the present report we demonstrate that the cyclopentenone prostaglandins PGA₁ and PGJ₂ at nontoxic concentrations can dramatically suppress HIV-1 replication during acute infection in CEM-SS cells. PGs did not affect HIV-1 adsorption, penetration, reverse transcriptase activity nor viral DNA accumulation in HIV-1 infected cells. A dramatic reduction in HIV-1 mRNA levels was detected up to 48–72 h after infection (p.i.) in PG-treated cells, and HIV-1 protein synthesis was greatly reduced by a single PG-treatment up to 96 h p.i. Repeated PGA₁-treatments were effective in protecting CEM-SS cells by the cytopathic effect of the virus, and in dramatically reducing HIV-1 RNA levels up to 7 d after infection. The antiviral effect was not mediated by alterations in the expression of α-, β-, or γ-interferon, TNFα, TNFβ, IL6, and IL10 in HIV-infected CEM-SS cells. The fact that prostaglandins are used clinically in the treatment of several diseases, suggests a potential use of cyclopentenone PGs in the treatment of HIV-infection. (J. Clin. Invest. 1996. 97:1795–1803.) Key words: antiviral • AZT • heat shock proteins • HIV-1 • prostaglandins

Introduction

Since the discovery of the human immunodeficiency virus (HIV) as the causative agent of acquired immune deficiency syndrome (AIDS), a number of compounds with antiretroviral activity have been identified (1). Although there has been considerable progress both in the understanding of the complex and multifactorial regulation of HIV replicative strategy, and in the management of chronic HIV infection, at present no effective treatment exists. Antiretroviral treatment with zidovudine or 2’, 3’-dideoxyinosine and 2’, 3’-dideoxycytidine, which inhibit reverse transcriptase activity (1), has limited efficacy in patients treated for prolonged periods (2), and the search for new antiviral drugs effective on different targets of HIV replication is needed to improve the therapeutic efficacy in HIV-infection and impair the progression of the disease.

The ability of prostaglandins (PGs) of the A type (PGAs) to inhibit virus replication and prevent the establishment of persistent infections was first reported in 1980 (3). It is now well established that PGs containing an α, β-unsaturated carbonyl group in the cyclopentane ring structure (cyclopentenone PGs, i.e. PGAs and PGJs) possess a potent antiviral activity against a wide variety of DNA and RNA viruses, including herpesviruses (4, 5), poxviruses (6), paramyxoviruses (3, 7), orthomyxoviruses (8), picornaviruses (9), togaviruses (10) and rhabdoviruses (11, 12). The antiviral activity of a long acting synthetic analogue of PGA₁, 16, 16-dimethyl-PGA₂ methyl ester (di-M-PGA₂) has been reported in vivo, in a mouse model infected with influenza A virus (8).

Prostaglandins of the A type have also been shown to inhibit the replication of retroviruses. PGA₁ was able to prevent the clonal selection of human T cell leukemia virus-type 1 (HTLV-1)-infected cord blood-derived mononuclear cells (13). Ankel et al. (14) reported that PGA₁ and PGA₂, but not PGB₁, PGE₁, and PGE₂, dramatically inhibited the replication of human immunodeficiency virus-type 1 (HIV-1) in C8166 cells. More recently, Hughes-Fulford et al. (4) have shown the antiviral efficacy of the PGA₁ analogue, dimethyl PGA₁, against HIV-1 in acutely infected T-lymphoma VB cells and in chronically infected macrophages. Higher concentrations of the drug were necessary to reduce p24 antigen levels in chronically infected cells as compared with acutely infected cells (4). Both authors suggested a possible use of PGAs or their synthetic analogs as antiviral agents in humans, specifically in the treatment of AIDS.

The mechanism of the antiviral activity of cyclopentenone prostaglandins has been mainly studied in negative-strand RNA virus models. In paramyxoviruses and rhabdoviruses the antiviral activity is mediated by alterations in the synthesis, maturation and intracellular translocation of specific virus proteins, and has been associated with the induction of the synthesis of heat shock proteins (HSP) 1 from the host cell (reviewed in reference 15). However, the mechanism of PGA₁ antiviral activity in cells infected with retroviruses is unknown.

In the present report we have studied the effect of the cyclopentenone prostaglandins PGA₁ and PGJ₁ on HIV-1 replication in CEM-SS cells during acute infection. Both prostaglandins were found to be extremely effective in inhibiting HIV-1 replication in these cells. The antiviral activity was not due to alterations in the adsorption and penetration of the virus into the host cell, nor to an effect on an early event in HIV-1...
replication cycle. A dramatic block of HIV-1 RNA expression was instead detected in cells treated with cyclophosphamide PGs.

Methods

Cell culture and virus. CEM-SS cells, derived from the human T lymphoid cell line CEM that expresses high levels of T4 antigen (16) were maintained at the density of 5 × 10^6 cells/ml in suspension medium RPMI 1640 (GIBCO BRL, Gaithersburg, MD) containing 2 mM L-glutamine, 10% heat-inactivated fetal calf serum (FCS, Hyclone Europe Ltd, UK) and antibiotics at 37°C in a 5% CO₂ atmosphere. H9 cells, a human CD4+ lymphocyte line (17), chronically infected with HIV-1, and the CD4+ human T cell line C8166, were grown in RPMI 1640 medium as described above. PGA, and PGJ2 (Cayman Chemical Co., Ann Arbor, MI) were stored as 100% ethanolic stock solutions at −20°C, and were diluted to the appropriate concentration in culture medium immediately before use. Azidothymidine (AZT; Sigma Chemical Co., St. Louis, MO) was dissolved in fresh RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (Hyclone), glutamine and antibiotics. PGA, and PGJ2 were maintained at the density of 5 × 10^6 cells/ml in suspension medium RPMI 1640 (GIBCO BRL, Gaithersburg, MD) containing 2 mM L-glutamine, 10% heat-inactivated fetal calf serum (FCS, Hyclone), and antibiotics at 37°C in a 5% CO₂ atmosphere. H9 cells, a human CD4+ lymphocyte line (17), chronically infected with HIV-1, and the CD4+ human T cell line C8166, were grown in RPMI 1640 medium as described above. PGA, and PGJ2 (Cayman Chemical Co., Ann Arbor, MI) were stored as 100% ethanolic stock solutions at −20°C, and were diluted to the appropriate concentration in culture medium immediately before use. Azidothymidine (AZT; Sigma Chemical Co., St. Louis, MO) was dissolved in PBS and used at the final concentrations of 5 μM.

HIV-1, isolate HTLV-IIIb, (17) was used in this study. An HIV-1 stock with a high infectivity was prepared by coculturing CEM-SS cells pretreated with Polybrene (8 μg/ml; Sigma Chemical Co.) for 12 h, with chronically infected H9 cells (3:1 ratio). At the time of appearance of syncytia, the culture medium was changed and virus-containing supernatant was harvested after 24 or 48 h. After clarification at 2000 rpm for 20 min and filtration, the titer of the viral stock was determined by vital dye exclusion technique.

Southern blot analysis. Uninfected or HIV-1 infected CEM-SS cells (1 × 10^6) were lysed in 100 mM NaCl, 10 mM Tris HCl pH 8.2, 25 mM EDTA pH 8, 0.5% SDS and 100 μg/ml proteinase K (18 h at 50°C), and DNA was isolated as described previously (21). Isolated DNA (70 μg) was digested for 3 h at 37°C with EcoRI (200 U) and BamHI (200 U) which recognize restriction sites within the HIV-1 genome at nucleotides 4647 and 5742 (EcoRI) and 8474 (BamHI) respectively. Digested DNA was then electrophoresed on a 1% agarose gel, transferred to Hybond-N nylon membranes (Amersham), and hybridized at 65°C with the pUCF12-HIV-1 plasmid (5 × 10⁵ copies/ml), labeled with α-32PdCTP (Amersham) by random priming (Random Primed DNA labeling kit; Boehringer Mannheim GmbH, Mannheim, Germany), in 5 × SSC, 5 × Denhardt’s solution, 0.5% SDS, 20 μg/ml denatured fragmented salmon sperm DNA. Blots were then processed for autoradiography, as described above. Two HIV-1 DNA fragments of ~1.100 and 2.700 bp were identified with this procedure, as expected (22).

DNA, RNA and protein synthesis. DNA, RNA and protein synthesis were determined in CEM-SS cells after treatment with PGA (4 μg/ml) or control diluent. Cells were labeled for a period of 24 h with 5 μCi/ml per 1 × 10⁶ cells of [3H]thymidine, [3H]uridine, or [35S]methionine (Amersham International, Buckinghamshire, England) for DNA, RNA or protein synthesis respectively, and the radioactivity incorporated into acid-soluble and -insoluble material was determined. Briefly, cells were washed three times with PBS, and 5% TCA (0.4 ml) was added to each culture. After 1 h at 4°C, the radioactivity of acid-soluble material was determined. Acid-insoluble radioactivity was measured after washing three times the TCA precipitates with ethanol, drying under an infrared lamp and dissolving the samples in 0.4 ml of a solution containing 0.1 M NaOH, 0.5% SDS.

Results

Anti-HIV activity of PGA in CEM-SS cells. CEM-SS cells were infected with HIV-1 (0.1 TCID₅₀/cell) and treated with different concentrations of PGA, or control diluent soon after the 1 h adsorption period. In the absence of PGA, HIV-1 was highly cytopathic for CEM-SS, causing syncytia formation at 48 h p.i., and cell death 7 d after virus infection. PGA treatment substantially decreased the number and size of virus-induced syncytia and prolonged for several days (3–4 d) the survival of infected cells.

Virus release from untreated and PGA-treated infected
cells was assessed at different times p.i. by determining HIV-1 p24 antigen production and the titer of infectious virus in the supernatant of infected cells. As shown in Fig. 1 A, PGA₁ treatment caused a dose-dependent decrease in p24 antigen production 96 h p.i., and an inhibition of p~ 90% was obtained at concentrations of 2 – 4 μg/ml, while higher concentrations of PGA₁ almost completely abolished p24 antigen production. The titer of infectious virus in the supernatant was also dramatically decreased in PGA₁-treated cells, and an inhibition of more than 4000-fold was detected in samples treated with 4 μg/ml PGA₁ at 72 h p.i., at which time p24 antigen production was decreased by approximately 80% in the same samples (Fig. 1 A, inset).

The concentration of 4 μg/ml was found to be the most active non toxic dose, not altering cell viability or RNA and DNA synthesis in uninfected CEM-SS cells (see below) and was used in all the following experiments. A single treatment with PGA₁ (4 μg/ml) started soon after the 1 h adsorption period, resulted in a sustained inhibition of virus production up to 7 d p.i. (Fig. 1 B). The antiviral activity was accompanied by a protection of CEM-SS cells by the cytopathic effect of the virus, as shown by determining the number of viable cells in uninfected or HIV-1 infected cells treated with PGA₁ or control diluent (Fig. 1 C). At 96 h p.i., the number of viable cells in HIV-1 infected cultures increased from less than 50% in untreated cells to > 90% in PGA₁-treated cells.

Under the conditions described above, PGA₁ at the concentration of 4 μg/ml was not toxic to uninfected CEM-SS cells as determined by microscopic examination and vital dye uptake (Fig. 2). As previously described in different cell lines (24), PGA₁ caused a moderate inhibition of cell proliferation at 48 h after the beginning of treatment, after which time cell proliferation rate went back to the normal level, unless the treatment was repeated (data not shown). A 24 h PGA₁-treatment also did not inhibit RNA or DNA synthesis in uninfected CEM-SS cells (Fig. 2).

To investigate the effect of PGA₁-treatment after infection with different concentrations of HIV-1, CEM-SS cells were infected with HIV-1 at 0.01, 0.1, 0.5, and 1 TCID₀/ cell and, after

Figure 1. Inhibition of HIV-1 replication by PGA₁ in CEM-SS cells. (A) CEM-SS cells infected with HIV-1 were treated with different concentrations of PGA₁ or control diluent soon after the 1 h adsorption period. P24 antigen production was determined 96 h p.i. by ELISA. Results are expressed as percent of p24 antigen production in untreated control (control = 152.0 ± 9.3 ng/ml). Inhibition of infectious virus production by PGA₁ (4 μg/ml; hatched bar) at 72 h p.i. is shown in A, inset. (B) Effect of PGA₁ (4 μg/ml) on extracellular (●) or intracellular (▲) p24 antigen production at different times after HIV-1 infection. (C) Effect of PGA₁ on HIV-1 cytopathic effect on day 2 and 4 p.i. Data represent the ratio between viable cells counts in infected cultures and viable cell counts in uninfected cultures for each time point. (○) control, (●) PGA₁ (4 μg/ml). SD < 10% are not shown.

Figure 2. Effect of PGA₁ on the proliferation and nucleic acid synthesis in uninfected CEM-SS cells. A. B. CEM-SS cells (5 × 10⁵ cells/ml) were treated with PGA₁ (4 μg/ml, hatched bar) or control diluent (open bar). Numbers of cells (A) and percent viability (B) were determined after 48 h. C-F. CEM-SS cells (1 × 10⁵ cells/ml) treated with PGA₁ (4 μg/ml, hatched bar) or control diluent (open bar) were labeled with [³H]thymidine (C and D) or [³H]uridine (E and F), and the amount of radioactivity incorporated into the TCA-soluble (uptake; C and E) or -insoluble (incorporation; D and F) material was determined after 24 h. Data represent the mean ± SD of duplicate samples (P > 0.05).
the 1 h adsorption period, the viral inoculum was removed and cells were treated with PGA (4 μg/ml) or control diluent. P24 antigen accumulation and the titer of infectious virus were determined in the supernatant of infected cells 96 h p.i.. Both p24 accumulation and infectious virus production increased in relation to the infectious dose (Fig. 3, A and B). PGA was found to dramatically inhibit HIV-1 replication, independently of the infectious dose.

**PGA does not affect an early event in HIV-1 replication cycle.** To determine whether PGA was acting on an early or a late event during HIV-1 replication cycle, CEM-SS cells were infected with HIV-1 (1 TCID<sub>50</sub>/cell) and treated with 4 μg/ml PGA soon after the 1 h adsorption period or at different times p.i. P24 antigen production was determined 96 h p.i. Fig. 4A shows that treatment with PGA started as late as 24 h p.i., was still effective in inhibiting p24 antigen production by more than 90% of control, indicating that PGA did not affect an early event in the virus cycle. In fact, PGA was able to moderately (~ 50%) inhibit p24 antigen production, even when treatment was started as late as 48 h p.i. Addition of PGA (4 μg/ml) to CEM-SS cells only during the virus adsorption period (1 h) did not inhibit HIV-1 replication (p24 antigen at 96 h p.i.: control = 174.0±9.6 ng/ml; PGA = 202.0±9.8 ng/ml), indicating that PGA did not affect virus adsorption to the cells. Moreover, PGA, even at much higher concentrations (50 μg/ml), did not alter HIV-1 reverse transcriptase (RT) activity when added directly to the reaction mixture during a RT in vitro assay (Fig. 4 B).

The effect of PGA treatment on viral DNA accumulation was also investigated. CEM-SS cells were infected with HIV-1 (10 TCID<sub>50</sub>/cell) and treated with PGA (4 μg/ml) or control diluent, soon after the 1 h adsorption period. At 24 h p.i., the accumulation of HIV-1 DNA was analyzed by Southern hybridization, using the full length HIV-1 probe, pUCF12-HIV-1 (20), after digestion with EcoRI and BamHI, as described in Methods. As shown in Fig. 5, digestion of DNA from HIV-1-infected cells by EcoRI and BamHI resulted in the detection of 2 fragments of 2.7 and 1.1 kb, which represent internal HIV-1 fragments between the EcoRI and the BamHI digestion sites. Higher molecular weight fragments (between 4.5 and 9 kb) correspond to digestion within the provirus and the flanking sequences of the human DNA. The results indicate that treatment with PGA had no effect on viral DNA accumulation in HIV-1 infected cells (Fig. 5). The effect of PGA treatment on HIV-1 yield was evaluated in the same experiment 96 h p.i., by determining p24 antigen production (C = 192.0±1.1 ng/ml; PGA = 34.0±2.7 ng/ml).

**PGA inhibits HIV-1 RNA transcription.** To investigate the effect of PGA on virus transcription, CEM-SS cells were infected with HIV-1 (1 TCID<sub>50</sub>/cell) and treated with PGA (4 μg/ml) or control diluent, soon after the 1 h adsorption period. Uninfected cells were treated identically. At 48 h p.i. total RNA was extracted from uninfected and HIV-1 infected cells and analyzed by Northern blot hybridization using a 3P-labeled full-length HIV-1 probe or a GAPDH probe as a control. At this time p.i. three major bands of HIV-RNAs could be detected in untreated infected cells corresponding to the non-spliced RNAs (9 kb), the envelope transcripts (4.2 kb) and the doubly-spliced, nonstructural mRNAs (2 kb), respectively (25). As expected, the accumulation of the 9-kb band, representing the viral genomic RNA and the mRNA for the gag and pol genes, was not as abundant as the two other bands, coding for viral proteins. None of the HIV-1 RNA bands could be observed in PGA-treated cells, indicating that the expression of HIV-1 provirus is dramatically inhibited in the presence of PGA (Fig. 6).

**Effect of multiple PGA treatments on HIV-1 RNA transcription.** To determine the effect of single or multiple PGA treatments on HIV-1 RNA transcription in long-term experiments, CEM-SS cells were infected with HIV-1 (1 TCID<sub>50</sub>/cell) and treated with PGA or control diluent soon after the 1 h adsorption period. In some samples treatment with PGA was repeated on day 3 and 6 p.i. Total RNA was extracted on day 2, 3, 5 and 7 after infection, and processed for Northern blot analysis (5 μg/sample), as described above. As shown in Fig. 7, also under these conditions, a single PGA<sub>1</sub>-treatment completely suppressed HIV-1 RNA expression up to 48 h p.i. and greatly reduced it even at 72 h p.i. At 5 and 7 d p.i. there was no difference in the level of HIV-1 RNA accumulation between control cells and cells which received a single PGA<sub>1</sub>-treatment, indicating that the block of viral RNA transcription by PGA is reversible, and is not due to a generalized toxic effect of PGA. However, re-addition of PGA to infected cells at day 3 and 6 p.i., resulted in a complete block of HIV-1 RNA transcription even at 5 d after infection, and HIV-1 RNA levels were still reduced at 7 d p.i., as compared to control. These results, together with the finding that treatment with PGA started as late as 24 h p.i. was effective in reducing HIV-1 mRNA levels.

**Figure 3.** The antiviral effect of PGA is independent of the infectious dose. CEM-SS cells were infected with HIV-1 at 0.01, 0.1, 0.5, and 1 TCID<sub>50</sub>/cell and treated with PGA (4 μg/ml; ○) or control diluent (○) soon after the 1 h adsorption period. P24 antigen production (A) and virus yield (B) were determined 96 h p.i.
for the following 48 h (data not shown), suggest that HIV-1 RNA transcription could be inhibited by PGA1 treatment even in established infections.

**Effect of PGA1 treatment on HIV-1 protein synthesis.** Prostaglandins of the A type have been shown to dramatically reduce the synthesis of virus proteins during acute infection with different negative-strand RNA viruses (26, 27). To investigate the effect of PGA1-treatment on HIV-1 protein synthesis, CEM-SS cells were infected with HIV-1 (1 TCID50/cell) and treated with PGA1 (4 μg/ml) or control diluent soon after the 1 h adsorption period. Treatment was not repeated in the following 96 h. At different times, the intracellular levels of viral proteins were analyzed by immunoblot analysis with anti-HIV-1 or anti-p24 gag antibodies. As shown in Fig. 8, the levels of intracellular p24 antigen were dramatically reduced up to 96 h p.i., as determined by ELISA or by Western blot analysis. At this time after infection, PGA1 only moderately inhibited total protein synthesis in HIV-1–infected cells, as determined by [35S]methionine incorporation (5 μCi/10⁶ cells, 6 h pulse). The synthesis of all HIV-1 virus proteins was inhibited by a single PGA1 treatment up to 96 h p.i., as determined by immunoblot analysis using polyclonal anti-HIV-1 antibodies, followed by ECL (data not shown).

**PGA1 does not induce the expression of cytokines in HIV-1 infected cells.** It has been reported that specific cytokines may modulate HIV-1 infection in cells of both the monocyte/macrophage and the lymphocyte lineage (28). To determine whether PGA1 treatment could modulate cytokine expression during HIV-1 replication cycle, infected CEM-SS cells were treated with 4 μg/ml or control diluent, soon after the 1 h adsorption period. At different time intervals, total RNA was extracted from PGA1-treated or untreated cultures, and analyzed by Northern blot, using 32P-labeled specific oligonucleotides for IL-6, IL-10, TNF-α, TNF-β, α-IFN, β-IFN, and γ-IFN. Under the conditions examined, high levels of TNF-β mRNA were found to accumulate in HIV-1 infected CEM-SS cells up to 96 h p.i., while low levels of TNF-α, γ-IFN, IL-10, and IL-6 were omitted.

**Figure 4.** PGA1 does not inhibit an early event in HIV-1 replication cycle. (A) CEM-SS cells were infected with HIV-1 and treated with control diluent (open bar) or PGA1 (4 μg/ml; hatched bar) soon after the 1 h adsorption period (time 0) or at 3, 6, 12, 24, and 48 h p.i. P24 antigen production was determined 96 h p.i. Results are expressed as percent of p24 antigen production in untreated control (control = 165.0±9.5 ng/ml) (SD < 10% are not shown). (B) Effect of PGA1 (●) on HIV-1 reverse transcriptase activity in vitro. (■) Control (no HIV-1 added). Data represent the mean±SD of triplicate samples.

**Figure 5.** Effect of PGA1 on HIV-1 DNA accumulation. CEM-SS cells were infected with HIV-1 (10 TCID50/cell) and treated with PGA1 (+) or control diluent (−) soon after the 1 h adsorption period. Uninfected cells (U) received the same amount of control diluent. At 24 h p.i. cells were harvested (10⁶ cells/sample) and genomic DNA was isolated, restricted with EcoRI and BamHI, and analyzed by Southern hybridization using 32P-labeled pUCF12-HIV-1 plasmid, as described in Methods. The 2.7- and 1.1-kb fragments represent the internal HIV-1 fragments between the EcoRI and BamHI digestion sites. Higher molecular weight fragments correspond to digestion within the provirus and the flanking sequences of the human DNA.

**Figure 6.** Inhibition of HIV-1 RNA expression by PGA1. Uninfected (U) or HIV-infected (HIV-1) CEM-SS cells were treated with 4 μg/ml PGA1 (+) or control diluent (−) soon after the 1 h adsorption period. At 48 h p.i. total RNA was extracted and analyzed by Northern blotting using the 32P-labeled pUCF12-HIV-1 probe. Levels of GAPDH mRNA in the same samples are shown as control.
the cytokines examined were found between PGA- and untreated. No difference in the levels of accumulation of any of the cytokines examined were found between PGAg-treated and untreated cells, up to 96 h p.i. (data not shown).

**Effect of PGJ2 on HIV-1 replication in CEM-SS cells.** Prostaglandins of the J type have been shown to potently suppress the replication of several RNA viruses (10, 26, 27, 29). To investigate the effect of PGJ2 on HIV-1 replication, CEM-SS cells were infected with HIV-1 (1 TCID50/cell) and treated with different concentrations of PGJ2 or control diluent soon after the 1 h adsorption period. Virus production from untreated and PGJ2-treated infected cells was determined 4 d p.i., measuring p24 extracellular levels by ELISA. As shown in Fig. 9 A, PGJ2 treatment caused a dose-dependent decrease in p24 antigen production; doses as low as 1.5–2 μg/ml inhibited p24 antigen production by > 90% as compared with control, while higher concentrations almost completely abolished p24 antigen production. To investigate whether PGJ2 treatment was also effective in inhibiting HIV-1 RNA transcription, CEM-SS cells were infected with HIV-1 and treated with PGJ2 (2 μg/ml) or control diluent, as described above. Total RNA was extracted from untreated or PGJ2-treated HIV-1 infected cells at 48 h p.i., and processed for Northern blot analysis. As described above for PGAg, PGJ2 treatment was found to dramatically inhibit HIV-1 mRNA expression in CEM-SS cells (Fig. 9 B).

Finally, the antiviral activity of PGAg and PGJ2 was compared with the effect of AZT-treatment in the same cells. CEM-SS cells were infected with HIV-1 (1 TCID50/cell) and treated with AZT (5 μM), PGAg (2.5, 5 or 10 μM) or control diluent, soon after the 1 h adsorption period. In a separate experiment, CEM-SS cells were infected with HIV-1 (1 TCID50/cell) and treated with 5 μM AZT, 4 μM PGJ2, or control diluent, as described above. After 96 h cell supernatants were collected and analyzed for p24 antigen accumulation. As shown in Fig. 10, the antiviral activity of PGAg and PGJ2 was comparable with that of AZT treatment in these cells.

**Discussion**

Prostaglandins are a class of naturally occurring cyclic 20-carbon fatty acids with potent biological properties. In eukaryotic cells, they are synthesized from arachidonic acid and other polyunsaturated fatty acid precursors derived from the phospholipid pool of the cell membrane in response to external stimuli, such as cell injury and inflammation (30) and function as intracellular signal mediators in the regulation of physiological and pathological processes, including inflammation and the febrile response (31, 32), cytoprotection (33), cell proliferation and differentiation (34) and virus replication (3, 15).

The antiviral activity of natural and synthetic arachidonic acid-derived cyclopentenones has been described in several DNA and RNA virus models in vitro and in vivo, at concentrations which are nontoxic to the host cell. Compared with other chemotherapeutic agents, the antiviral activity of cyclopentenone PGs is characterized by a wide spectrum of action, since it affects both naked or enveloped DNA and RNA viruses,
Inhibition of HIV-1 replication by cyclopentenone prostaglandins is comparable to AZT treatment. (A) CEM-SS cells were infected with HIV-1 (1 TCID<sub>50</sub>/cell) and treated with 5 μM AZT (bar 2), 2.5, 5 or 10 μM PGA<sub>1</sub> (bars 3, 4, and 5, respectively) or control diluent (bar 1), soon after the 1 h adsorption period. (B) In a separate experiment, CEM-SS cells were infected with HIV-1 (1 TCID<sub>50</sub>/cell) and treated with 5 μM AZT (bar 2), 4 μM PGJ<sub>2</sub> (bar 3), or control diluent (bar 1) as described above. P24 antigen production was determined 96 h p.i. by ELISA.

The mechanism of the antiviral activity is not completely elucidated. Cyclopentenone PGs appear to act at more than one level during the virus replication cycle. In several virus models, the target for the antiviral activity is a late event in the virus replication cycle, and PGs cause alterations in the synthesis of the virus-induced shut-off of cellular protein synthesis and in the protection of the host cell from the virus-induced shut-off of cellular protein synthesis (26, 27). This block has been shown to be exerted at the translational level, and is dependent upon the induction of a 70-kD heat shock protein (hsp70) in infected cells (15, 26).

Prostaglandins with antiviral activity, in fact, function as a signal for the induction of heat shock proteins (HSP) and in particular of hsp70 in a large variety of mammalian and human cells lines, as well as in human peripheral blood lymphocytes, macrophages and primary cells derived from cord blood (24, 37). Induction of hsp70 gene transcription by prostaglandin A is mediated by cycloheximide-sensitive activation of heat shock transcription factor (HSF), which binds to the heat shock element composed of multiple adjacent inverted repeats of the pentamer nGAAn (38), and it has been recently associated with the antiviral activity of these molecules (reviewed in reference 15).

In the present study, we have demonstrated that two cyclopentenone prostaglandins, PGA<sub>1</sub> and PGJ<sub>2</sub>, are extremely effective in suppressing HIV-1 replication during acute infection in lymphoblastoid CEM-SS cells. A 10-fold reduction of p24 antigen production, and an over 1000-fold reduction in infectious virus yield, as determined by infectious virus titration, can be obtained at concentrations of prostaglandins which are not toxic to the cells and do not inhibit nucleic acid synthesis in uninfected CEM-SS cells. The antiviral activity was found to be comparable to that of AZT treatment in these cells.

Prostaglandins did not affect the adsorption and penetration of the virus into the host cell, nor an early event in HIV-1 replication cycle, since PGA<sub>1</sub> was found not to alter the accumulation of viral DNA up to 24 h p.i., and treatment started as late as 24 h p.i. was still effective in inhibiting virus replication. HIV-1 reverse transcriptase activity was also unaffected by PGA<sub>1</sub>. Both PGA<sub>1</sub> and PGJ<sub>2</sub> were found to dramatically suppress HIV-1 mRNA expression in these cells up to 48–72 h p.i. Synthesis of HIV-1 proteins was consequently inhibited up to 96 h after infection. Delayed (up to 24 h p.i.) and repeated PGA<sub>1</sub>-treatments were effective in protecting CEM-SS cells by the cytopathic effect of the virus, and in reducing HIV-1 RNA levels up to 7 d after infection, suggesting that HIV-1 RNA transcription could be inhibited also in established infections.

The inhibition of HIV-mRNA expression does not appear to be mediated by different cytokines, including α, β, or γ-interferon, TNF-α, TNF-β, IL-6, or IL-10, whose expression is not altered by PGA<sub>1</sub> in HIV-1-infected CEM-SS cells. Cyclopentenone prostaglandins may be affecting other intracellular signalling pathways used by HIV-1 to regulate its expression, including activation of the transcription factor NF-κB (39), or the synthesis of heat shock proteins and cyclophilins, whose role in the control of HIV-1 life cycle has been recently described (reviewed in reference 40). PGA<sub>1</sub> has in fact been shown to induce the expression of an oxidative stress-regulated protein, heme oxygenase (41), as well as other heat shock proteins (15, 24, 37). As described above, hsp70 has been shown to participate in the PGA<sub>1</sub>-induced block of virus protein translation in cells infected with negative-strand RNA viruses (26). Since PGA<sub>1</sub> is able to induce the synthesis of elevated intracellular levels of hsp70 in HIV-1-infected CEM-SS cells up to 24 h after treatment (De Marco et al., manuscript in preparation), it could be hypothesized that, also in the case of retrovirus infection, hsp70 could interfere with virus mRNA transcription directly, or by inhibiting the translation of the trans-acting regulatory HIV-1 proteins Tat or Rev, which would in turn result in a block of HIV-1 gene expression (42).

Based on the results obtained in other virus models, we cannot exclude that PGA<sub>1</sub> is acting at more than one level during HIV-1 replication cycle. In fact, the finding that in the same samples the production of p24 antigen is reduced by ~10-fold, while the virus yield, as measured by titration of infectious virus, is reduced by over 1000-fold by PGA<sub>1</sub>-treatment, indicates that not only PGA<sub>1</sub> inhibits HIV-1 production...
by infected cells, but that a large amount of the virus particles produced are not infectious. This suggests that HIV-1 glycoprotein maturation and intracellular translocation could also be affected by PGA₁, as previously shown for VSV G glycoprotein (11), and Sendai virus HN and F glycoproteins (36), and deserves further investigation.

It should be pointed out that concentrations of PGA₁ higher than 1 μM were necessary to inhibit HIV-1 replication in our in vitro model, indicating a pharmacological effect. Whether the level of cyclopentenone prostaglandins could achieve a sufficiently high local concentration to inhibit HIV-1 replication in vivo during pathological conditions that cause an increase in arachidonic acid metabolism, such as hyperthermia (43), remains to be established.

Prostaglandins are used clinically in the treatment of congenital heart disease and gastric ulcers, and to facilitate labor, and are generally effective and well tolerated. In studies on volunteers with hypertension, infusion with PGA₁ was reported to have a beneficial effect on the patients’ blood pressure (44, 45), while no deleterious effects on kidney function nor other significant side-effects were found. These observations, together with the progress in the understanding of the mechanism of antiviral action, encourage the search for new prostanoids with antiretroviral activity and make cyclopentenone prostaglandins a new class of potential therapeutic agents for HIV-1 infection.

Acknowledgments

We thank M. Federico for the pUCF12-HIV-1 plasmid.

This work was supported by a grant from the Italian Ministry of Public Health, VIII AIDS Research project (1995). A. Carattoli and A. De Marco were supported by a Fellowship for AIDS Research from the Italian Ministry of Public Health.

References


