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Antiviral effects of mifepristone on human immunodeficiency virus type-1 (HIV-1): Targeting Vpr and its cellular partner, the glucocorticoid receptor (GR)

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Abstract

The HIV-1 viral protein R, Vpr, increases virus replication in T cells and is necessary for the optimal infection of primary monocytes/macrophages and other non-dividing cells. Vpr interacts with the cellular glucocorticoid receptor (GR) and transactivates the HIV-1 LTR through glucocorticoid response element (GRE), an event that can be blocked by the GR antagonist, mifepristone. Results demonstrated that Vpr-induced transactivation of the HIV-1 LTR was inhibited by mifepristone in a dose-dependent manner by >60% at a 10 μM concentration. Infectivity assays using X4 and R5 viruses demonstrated antiviral effects on a dose-dependent regimen of mifepristone. The effects of mifepristone were also tested in latently infected cells that could be activated with extracellular Vpr protein and results indicated specific inhibition of virus reactivation in the presence of this antagonist.

Keywords: HIV-1; Antiviral; Vpr; Mifepristone; Glucocorticoid receptor

1. Introduction

There are currently an estimated 42 million people infected worldwide with Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS), with approximately 14,000 new infections occurring every day (UNAIDS/WHO statistics). While current therapies including highly active anti-retroviral drug therapy (HAART) have without doubt prolonged the lives of many fighting HIV and have prevented innumerable new infections, the drug regimen is costly and adherence is generally low (d'Arminio Monforte et al., 2000; Deloria-Knoll et al., 2004). Additionally, the development of resistance to available antiviral drugs is another major set back (Lucas, 2005; Sethi et al., 2003). Generally, HAART is administered as "triple therapy" consisting of a protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI) as well as two nucleoside reverse transcriptase inhibitors (NRTIs) (Pommier et al., 2005). Unfortunately, these therapies often lead to toxic side effects including mitochondrial toxicity, lipodystrophy, lactic acidosis, hepatatoxicity, and cardiomyopathy (Chariot et al., 1999; Friis-Moller et al., 2003; Montessori et al., 2004; Sulkowski et al., 2000). Currently, other antiviral targets are being explored including integrase inhibitors, CXCR4 and CCR5 inhibitors, and cellular proteins including APOBEC3G and TRIM5α that demonstrate antiviral activity (Baba et al., 1999; Donzella et al., 1998; Harris and Liddament, 2004; Richman, 2001; Stremlau et al., 2004; Yap et al., 2004).

Viral reservoirs such as macrophages and resting T cells prevent total viral clearance during HAART (Collman et al., 2003; Finzi et al., 1997; Lambotte et al., 2000; Wong et al., 1997). Though there is some speculation that patients treated with HAART <6 months post-seroconversion are able to decrease latent viral loads to less than detectable levels, it is hypothesized that patients would have to undergo 60 years of HAART to clear all viral reservoirs (Finzi et al., 1999; Strain et al., 2005). While there is great pursuit to find new therapies and vaccines, there is a substantial need to find additional novel compounds, which will target areas of virus replication not yet investigated for antiviral intervention.

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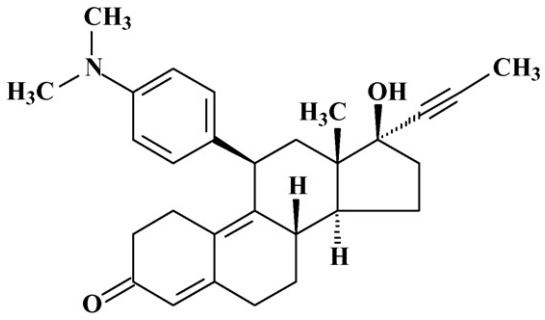

Fig. 1. Chemical structure of mifepristone.

The HIV-1 accessory protein, viral protein R (Vpr), is 96-amino acids in length with a molecular weight of approximately 14 kDa. Vpr can be defined as a pleiotropic protein, as it has been shown to mediate an array of varying functions including cell cycle arrest at the G2/M phase, immune regulation and evasion, apoptosis, transport of the pre-integration complex (PIC), and viral transactivation (Agostini et al., 1996; Ayyavoo et al., 1997, 2002; Fukumori et al., 2000; He et al., 1995; Jowett et al., 1995; Majumder et al., 2005; Popov et al., 1998; Wang et al., 1995). Vpr is also packaged within the virion, possibly transactivating viral genes prior to the production of Tat (Hrimech et al., 1999). Studies have shown that Vpr, while important during infection of dividing cells such as T cells, is vital to the optimal infection of non-dividing cells such as macrophages, due to its involvement with PIC (Connor et al., 1995; Hattori et al., 1990; Sherman et al., 2003). The importance of Vpr in the initial stages of infection, therefore, can be demonstrated by the necessity of Vpr for optimal macrophage infection during initial virus/host cell contact.

Vpr transactivates the HIV-1 LTR and increases virus replication in target cells (Agostini et al., 1996; Cohen et al., 1990; Wang et al., 1995). Specifically, HIV-1 Vpr-mediated transactivation is shown to occur in the presence of steroid receptors and other co-activators (Kino et al., 1999; Sherman et al., 2000; Thotala et al., 2004). Steroid receptors belong to a super family of ligand-dependent transcription factors that are known to interact with proteins containing the signature LxxLL motif (Feng et al., 1998; Heery et al., 1997). Thus this interaction suggests a possible new function for Vpr through utilization of the GR pathway thereby increasing viral transcription. Previously we demonstrated that immune dysregulation by Vpr was related to the ability of Vpr to suppress NF-kB activity through the induction of IkB transcription. This suppression occurred in a manner similar to that of glucocorticoid-mediated suppression and was abrogated by mifepristone (Fig. 1), a class II GR antagonist (Ayyavoo et al., 1997; Brogden et al., 1993). We hypothesize that targeting Vpr-GR complex-mediated transactivation could be beneficial in inhibiting Vpr-mediated viral and host cellular dysregulation. In this study, we have evaluated the ability of mifepristone to inhibit Vpr-mediated transactivation of the autologous promoter, HIV-1 LTR, as well as the antiviral activity of mifepristone against X4 and R5 viruses.

2. Materials and methods

2.1. Cell lines

HeLa, HEK293, and TZM-bl cell lines were grown in DMEM (Gibco, Carlsbad, CA) supplemented with 10% FBS (HyClone, Logan, UT), 1% Penicillin–Streptomycin, and 1% L-Glutamine. HeLa cells were obtained through the NIH AIDS Research and Reference Reagent program from Dr. Richard Axel (Maddon et al., 1986). TZM-bl cells were received from the AIDS Research and Reference Reagent program from Drs. John C Kappes, Xiaoyun Wu, and Tranzyme, Inc. (Derdeyn et al., 2000; Platt et al., 1998; Wei et al., 2002). HEK293 cells were from the NIH AIDS Research and Reference Reagent Program by Dr. Andrew Rice (Graham et al., 1977). U1/HIV-1 and 174 × CEM cell lines were grown in RPMI 1640 (Gibco) and supplemented with 10% FBS, 1% L-Glutamine, and 1% Penicillin–Streptomycin. 174 × CEM cells were provided by Dr. Peter Cresswell and were received from the AIDS Research and Reference Reagent Program (Salter et al., 1985). The U1/HIV-1 and OM-10.1 cells were acquired through the AIDS Research and Reference Reagent Program as contributed by Dr. Thomas Folks and Dr. Salvatore Butera, respectively.

Peripheral Blood Mononuclear Cells (PBMCs) were isolated using blood from healthy normal donors using Lymphocyte Separation Media (Mediatech, Herndon, Virginia) gradient centrifugation. PBMCs were resuspended in RPMI 1640 supplemented with 10% FBS (R10) and stimulated with phytohemoagglutinin (PHA-P) (Sigma, St. Louis, MO) (5 μg/mL) for 3 days. Post-stimulation, cells were cultured in R10 in the presence of rIL-2 (Chiron, Emoryville, CA) (5 U/mL). Monocyte-derived macrophages were isolated from PBMCs by adhesion as described (Collman et al., 1989). Briefly, PBMCs were resuspended in DMEM supplemented with 1% Penicillin–Streptomycin and 1% L-Glutamine without the presence of serum and were plated for 2 h at 37 °C, to allow the monocytes to adhere to the plates. After the 2-h incubation, non-adherent cells were removed and the adherent cells were washed two times in phosphate-buffered saline (PBS). DMEM supplemented with 10% FBS, 10% either horse or human sera, 1% Penicillin–Streptomycin, 1% L-Glutamine, 500 U/mL GM-CSF (granulocyte-macrophage colony stimulating factor) (Berlex Labs, Richmond, California), and 15 ng/mL M-CSF (macrophage colony stimulating factor) (Amgen, Thousand Oaks, CA) were added to the cells to begin the differentiation of the monocytes into macrophages. Half the media was replaced with an equal quantity of fresh media containing cytokines every 3 days.

2.2. Plasmids and viruses

For transactivation studies, the HIV-1 LTR luciferase reporter construct was obtained from the NIH AIDS Research and Reference Reagent Program from Dr. Reink Jeeninga and Dr. Ben Berkhout (Jeeninga et al., 2000; Klaver and Berkhout, 1994). The Vpr expression plasmid was constructed as described (Thotala et al., 2004). HIV-1 89.6 virus stock was received from

the NIH AIDS Research and Reference Reagent Program from contributor Dr. Ronald Collman (Collman et al., 1992). HIV-1 Ba-L was received from the NIH AIDS Research and Reference Program, contributed by Drs. Suzanne Gartner, Mikulas Popovic, and Robert Gallo (Gartner et al., 1986; Popovic et al., 1989). pNL43^{wt} was obtained from NIH AIDS Research and Reference Reagent Program contributed by Dr. Malcolm Martin (Adachi et al., 1986). pNL43-EGFP was a kind gift from Dr. David Levy (University of Alabama) and was constructed as described (Kutsch et al., 2002). For all viruses, viral titer was determined through p24 ELISA and the number of infectious particles was determined by TZM assay. TZM-bl cell line was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, from John C. Kappes, Xiaoyun Wu, and Tranzyme Inc. This indicator cell line is a HeLa cell line derivative that expresses high levels of CD4 and CCR5 along with endogenously expressed CXCR4. TZM-bl cells contain HIV long terminal repeat (LTR)-driven β-galactosidase and luciferase reporter cassettes that are activated by HIV *tat* expression (Sun et al., 2005).

2.3. Cytotoxicity assays

To determine the percent of cytotoxicity *in vitro* of cells in the presence of mifepristone, the MTT Tetrazolium Assay, developed by Mosmann (1983), was performed as described. HeLa or HEK293 cells were plated (15,000/well) in a 96-well plate overnight for adherence. Mifepristone was then administered in triplicate in serial dilutions of differing concentrations for 3 days in a total volume of 200 μL. Three days post incubation, MTT (3-(4,5,dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) dye solution (5 mg/mL in phosphate buffer pH 7.4) (Sigma, St. Louis, MO) was added (20 μL/well) for 4–5 h at 37 °C in 5% CO_2. After incubation with the MTT dye solution, cell media was removed and 200 μL of DMSO was added by flushing so that the MTT salt crystals would fully dissolve. The cells were incubated at 37 °C for 5 min and read at 490 nm. Similar analyses were performed for PBMCs and macrophages and results are presented as percent viability.

2.4. Effects of mifepristone on HIV-1 Vpr-mediated transactivation

HeLa cells were co-transfected in 6-well plates with the HIV-1 LTR-Luc (2.5 μg) reporter construct in the presence of pVpr or control vector. Mifepristone was added in a dose-dependent manner. Forty-eight hours post-transfection, cells were lysed in 1X Reporter Lysis Buffer (Promega, Madison, WI). Luciferase activity was measured by RLU and normalized by protein estimation.

2.5. Antiviral assays in primary cells and latently infected cells

To determine the anti-viral effects of mifepristone on infection, PBMCs were infected with an MOI of 0.1 or with 100 ng p24 equivalent virus for 6 h at 37 °C. Post infection, virus-containing media was removed and cells were washed twice in PBS and re-suspended in fresh IL-2-containing media. Mifepristone or the EtOH (vehicle control) was added in a dose-dependent manner. Supernatants (500 μL) were removed every 2–3 days at which time mifepristone or EtOH was re-administered due to the half-life (48–72 h) of the compound. Virus replication was monitored by measuring the p24 released into the media by ELISA (NIH, AIDS Vaccine Program, Frederick, MD). Similarly, macrophages were infected with an MOI of 0.1 or 0.5 of Ba-L virus and antiviral activity was evaluated as described above. To demonstrate the effects of mifepristone in an established cell line, 174 × CEM were infected with an MOI of 0.5 of the pNL43-EGFP reporter virus as described before and mifepristone was added in a dose-dependent manner to the cells. Post-treatment cells were fixed with 2% paraformaldehyde and analyzed by FACS at different intervals. Latently infected cells OM-10.1 and U1/HIV-1 were reactivated with recombinant Vpr or control protein (glutathione *S*-transferase, GST) as described (Levy et al., 1994; Nakamura et al., 2002) in the presence or absence of various concentrations of mifepristone and antiviral activity was assessed by measuring the p24 released into the medium.

3. Results

3.1. Cytotoxicity of mifepristone in target cells

In order to determine the toxicity of mifepristone *in vitro*, cytotoxicity (CT) assays were performed in the cell lines HeLa and HEK293, as well as primary PBMCs and monocyte-derived macrophages. Either standard MTT assay and/or trypan blue exclusion assays were used to determine CT_{25}, CT_{50}, and CT_{95} in these cells (Table 1). Results indicated that the concentration required to induce 50% cell death (CT_{50}) varied for each cell tested. For instance, the CT_{50} for normal donor peripheral lymphocytes was 35 μM, whereas for monocyte-derived macrophages it was >250 μM. Additionally, we also tested the CT_{50} in a number of cell lines including HeLa and HEK293T and the results indicated 50 and 40 μM, respectively, as CT_{50}. Among the cells tested, PBMCs were the most sensitive, whereas macrophages tolerated higher doses in long-term cultures suggesting that mifepristone exhibited a relatively low toxicity *in vitro* analysis.

Table 1
Cytotoxic effects of mifepristone determined by MTT assay

Cell line	CT25 (μM)	CT50 (μM)	CT95 (μM)
HeLa	18 ± 1.4	>50	>100
HEK293	11 ± 2.3	41.7 ± 7.6	>50
PBMC	9 ± 5.1	36 ± 3.2	>100
Macrophage	105 ± 16.6	295	>500

Cell lines HeLa and HEK293 and human primary PBMCs and monocyte-derived macrophages were assessed for cytotoxicity in the presence of mifepristone. Cells were exposed to different concentrations of mifepristone. Three days post-treatment cell death/viability was analyzed by MTT assay. Results reflect the average and S.D. of at least three independent experiments.

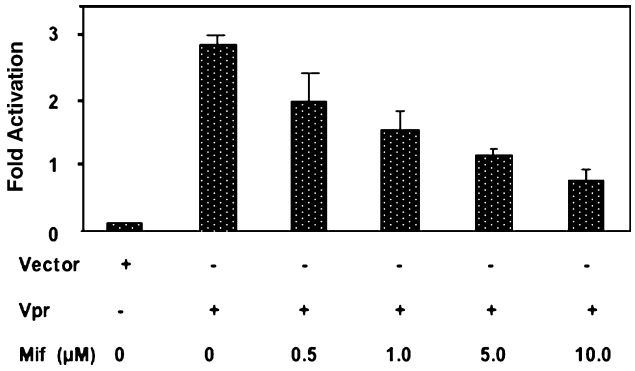

Fig. 2. Effects of mifepristone on Vpr-induced transactivation of HIV-1 LTR. HeLa cells were co-transfected with the luciferase reporter HIV-1 LTR-luciferase and pVpr expression plasmid or vector plasmid. Cells were maintained in the presence of various concentrations of mifepristone. Forty-eight hours post-transfection cells were lysed and luciferase activity was measured. Fold activation was calculated considering the vector transfected as 0. The fold activation S.D. was derived from three independent experiments.

3.2. Inhibition of HIV-1 Vpr-mediated promoter activity by mifepristone

HIV-1 Vpr, in conjunction with cellular GR, transactivates the HIV-1 LTR through GRE sequences present within the promoter region (Vanitharani et al., 2001). To test whether HIV-1 Vpr-induced LTR transactivation could be inhibited by the GR antagonist mifepristone, HeLa cells were co-transfected with the HIV-1 LTR-luciferase reporter plasmid and pVpr in the presence and absence of different concentrations of the GR antagonist, mifepristone (Fig. 2). Results demonstrated that presence of Vpr increase the HIV-1 LTR transactivation by 2–2.5 fold as shown before (Thotala et al., 2004), whereas mifepristone inhibited the transactivation of HIV-1 LTR-driven gene expression in HeLa cells in a dose-dependent manner. Mifepristone inhibited the transactivation by greater than 60% at a concentration of 10 μM, whereas the inhibition was 27% at 0.5 μM. Similar results were observed in other cell lines (data not shown), thereby demonstrating the effectiveness of this compound in decreasing Vpr-mediated transactivation of the viral promoter.

3.3. Inhibition of virus replication by mifepristone in CEM × 174 cells

Though HIV-1 Vpr is dispensable for virus replication in T cells, the presence of Vpr enhances virus replication (Levy et al., 1995; Rucker et al., 2004). In order to identify whether mifepristone blocks virus replication, we assessed the inhibitory activity of mifepristone in the established target cell line, CEM × 174. Utilizing the EGFP-expressing NL43 reporter virus, we infected CEM × 174 cells with an MOI of 0.5. Post-infection, mifepristone was added in a dose-dependent manner and cells were assessed for the expression of EGFP through FACS analysis (Fig. 3). At a MOI of 0.5, 53% of cells were infected at day 4 post-infection. When cells were maintained in the presence of 10 μM concentration of mifepristone, the percentage of EGFP-positive cells were reduced to 6% indicating an 88% reduction in infectivity compared to the infected/untreated culture or vehicle-treated (ethanol) culture. This effect was dose-dependent and a concentration of 0.5 μM was required to attain 50% inhibition. Furthermore, FACS analysis demonstrated little to no cell death in the presence of mifepristone at the 4-day time point, exemplifying that there were no toxic effects that played a role in the inhibition of viral replication. To further confirm these results, p24 samples were taken at the time of FACS analysis (data not shown). Results demonstrated by p24 exhibited an inverse correlation with a decreasing concentration of mifepristone.

3.4. Effects of mifepristone on productive virus replication in human primary PBMCs

To determine the antiviral effects of mifepristone in relevant primary cells that are natural targets for HIV-1, virus replication was assessed in the presence and absence of various concentrations of mifepristone in PHA-stimulated normal human PBMCs infected with the dual-tropic (X4 and R5) HIV-1 strain, 89.6. PHA-stimulated PBMCs were infected as described and mifepristone was added 6 h post-infection. Culture supernatant was collected every 2 or 3 days and fresh medium and compound were added. Virus replication was monitored by measuring the p24 antigen released into the medium (Fig. 4). Viral replication was completely abolished (>95%) at a concentration of 10 μM, whereas a 60–70% inhibitory effect was observed at a concentration of 1 μM indicating a dose-dependent effect. Similar results were observed in PBMCs derived from multiple donors ($N = 5$). Analysis of cell viability in these long-term cultures by trypan blue test and/or morphology changes shown in Fig. 4 panel C, indicated an increase in toxicity at the highest concentrations (10 μM) at 7 days post-infection, suggesting that the effect of mifepristone in these cultures were independent of apoptosis. Together these results suggest that mifepristone inhibits virus replication in a dose-dependent manner.

3.5. Effects of mifepristone on virus replication in monocyte-derived macrophages

Monocytes/macrophages and other non-dividing cells serve as initial cellular targets as well as latent reservoirs. To determine the effects of mifepristone on monocyte-derived macrophages, macrophages were infected with the CCR5-using Ba-L virus at an MOI of 0.1 or 0.5. Post-infection, cells were treated with different concentrations of mifepristone and virus replication was monitored as determined by p24 (Fig. 5). Results demonstrated a dose-dependent decrease in viral replication in the presence of mifepristone in two separate donors (Panels A and B) when infected with an MOI of 0.1. Infectivity was totally abolished at a concentration of 50 μM, whereas 50–70% inhibition was observed at a 10 μM concentration. Similar effects were observed when the MOI was increased to 0.5. However, the lower concentrations did not have any effect on virus replication. Since 10 μM was toxic to PBMCs, we tested the culture for cytopathicity and our results indicate that no cell death was observed at a 10 μM concentration 7 days post-treatment (Fig. 5, Panel C). This effect was seen throughout the entire culture period

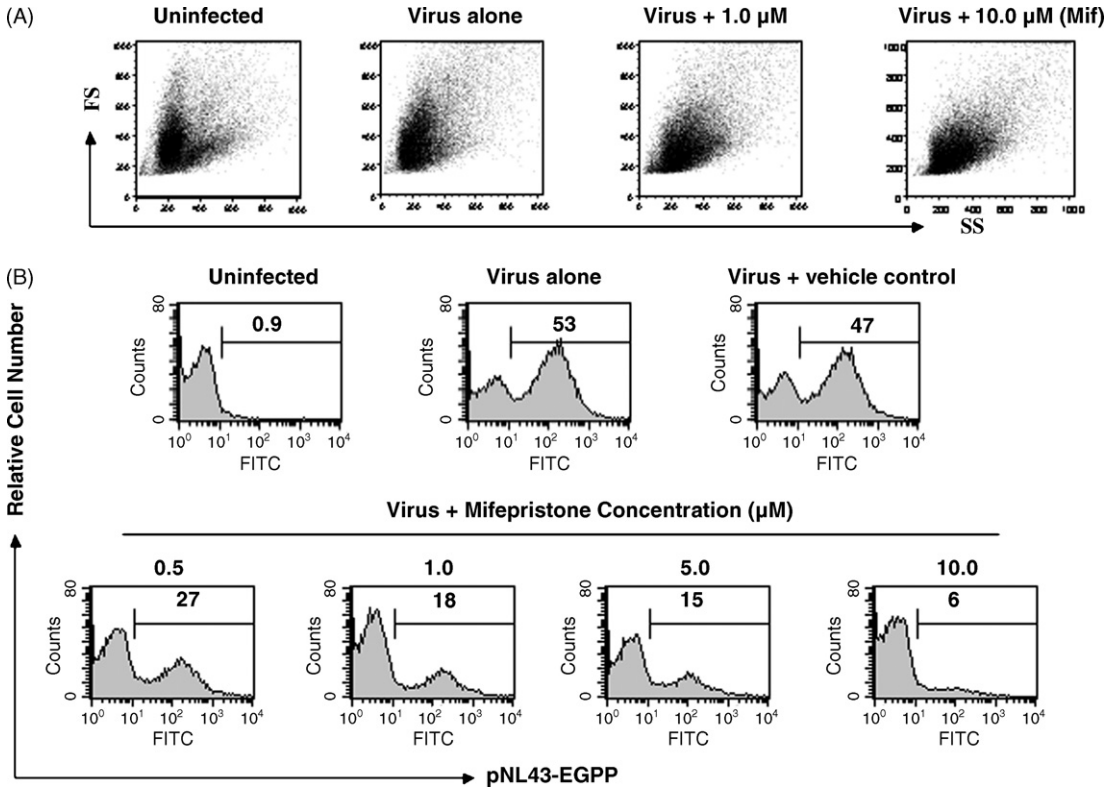

Fig. 3. Effects of mifepristone on NL43-EGFP infection as determined by FACS analysis. CEM × 174 cells were infected with 0.5 MOI of the EGFP-expressing pNL43 virus. Post-infection cells were maintained in media containing different concentrations of mifepristone. Cells were collected 3 days post-infection and analyzed by FACS analysis. (A) Forward and side scatter of the cells under different treatment to identify the live cell population; (B) percentage of EGFP positive cells that reflects the number of infected cells. Results are representative of two separate experiments. Uninfected denotes No Treatment; Virus alone, represents cells infected with virus; Vehicle Control represents infected cells in the presence of ethanol; Virus + Mif., denotes infected cells in the presence of different concentrations of mifepristone.

indicating that reinfection by the infected cells was suppressed by the presence of mifepristone in a dose-dependent manner.

3.6. Effects of mifepristone on virus reactivation in latently infected OM-10.1 and U1 cells

One of the mechanisms by which HIV-1 remains elusive to total viral clearance during therapy is due to its ability to establish viral reservoirs (Collman et al., 2003; Finzi et al., 1997; Lambotte et al., 2000; Wong et al., 1997). In order to determine if mifepristone was able to prevent reactivation from latency, the promonocytic cell line U1/HIV-1 was used. This cell line contains two copies of the proviral HIV-1 DNA which upon stimulation by the viral protein Vpr or other factors such as TNF-α, leads to the production of virus, which can be quantitated by p24 ELISA (Levy et al., 1994; Nakamura et al., 2002). To establish if mifepristone was able to prevent the reactivation of the proviral DNA contained within the U1/HIV-1 and OM-10.1 cells, cells were activated with purified recombinant Vpr protein or control GST protein in the presence or absence of different concentrations of mifepristone and virus production was evaluated (Fig. 6). Results demonstrated that activation of OM-10.1 (panel A) and U1/HIV-1 (panel B) cells by rVpr resulted in significant level of virus production, whereas control GST did not show any increase in p24 level above the basal level. As expected OM-10.1 cells produced significantly high levels of p24 compared to the defective U1 cells. Similarly, the dose required to inhibit ∼50% of virus production was 10 μM and 5 μM for OM-10.1 and U1 cells, respectively. Higher doses of mifepristone were required to inhibit >90% of virus production and at this level this compound was toxic to the cells. Additionally, when mifepristone was removed from the cells the inhibitory effect was eliminated indicating that presence of the active compound is necessary to control virus replication.

4. Discussion

Current antiviral regimens such as HAART, a breakthrough in HIV-1 antiviral treatment, eventually fail, leading to the onset of AIDS and ultimately death for those infected. With a better understanding of HIV-1 replication and the role of cellular proteins in HIV-1 pathogenesis, new classes of antivirals are being pursued and new drug treatment regimens are being discovered (Baba et al., 1999; Donzella et al., 1998; Harris and Liddament, 2004; Richman, 2001; Stremlau et al., 2004; Yap et al., 2004). Unfortunately, however, there has of yet been no proven regimen to fully combat this virus or target the hidden cellular viral reservoirs. Instead, virus resistance has been increasing, and many of those infected are quickly failing current therapies.

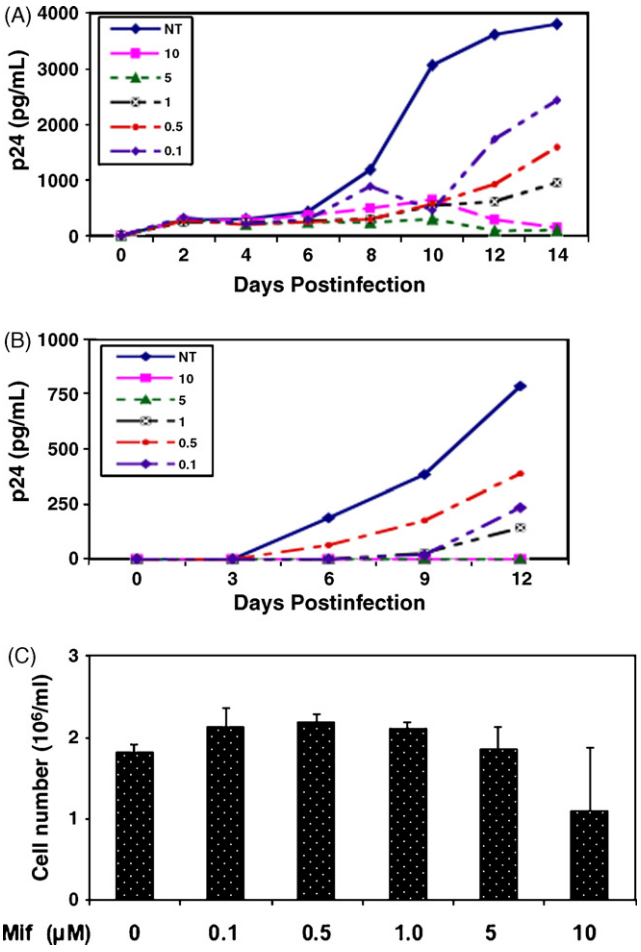

Fig. 4. Antiviral effect of mifepristone on 89.6 virus-infected PBMCs. (A and B) PBMCs were infected with an MOI of 0.1 of the dual-tropic (X4 and R5) 89.6. Post-infection, mifepristone was added in a dose-dependent manner and supernatant samples were collected every 2 or 3 days and analyzed for p24. These experiments were repeated using PBMC's from multiple seronegative donors ($N = 5$) and panels A and B represent the antiviral effect of mifepristone in PBMCs from two different donors. NT represents No Treatment. (C) Cell viability was determined by Trypan blue counting performed at day 7 post infection for various doses of mifepristone and S.D. was derived from three different donors.

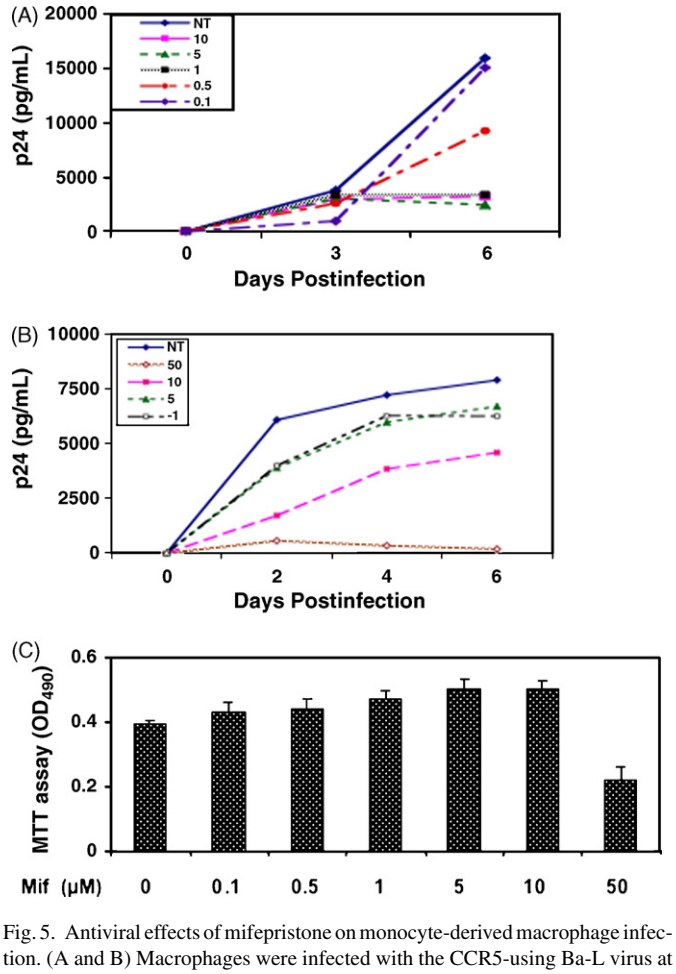

Fig. 5. Antiviral effects of mifepristone on monocyte-derived macrophage infection. (A and B) Macrophages were infected with the CCR5-using Ba-L virus at an MOI of 0.5. Post-infection, mifepristone was added in a dose-dependent manner and supernatant samples were collected every 2–3 days and analyzed for p24. These experiments were repeated using macrophages from different seronegative donors and panels D1 and D2 represent the antiviral effect of mifepristone in macrophages from two different donors as a representation. NT represents No Treatment. (C) Cell viability was determined by performing MTT assay on 7-day post infection for various doses of mifepristone and S.D. was derived from two different donors.

One of the HIV-1 accessory proteins, Vpr, has been implicated in a variety of functions including being a vital constituent of the pre-integration complex, controlling cellular functions, re-activating latent infection, and involvement in the infection of non-dividing target cells (Connor et al., 1995; Hattori et al., 1990; Sherman et al., 2003). Vpr is also known to interact with different cellular partner(s) including the glucocorticoid receptor. One of the means in which Vpr increases LTR-mediated transactivation is through its interaction with GR, leading to the transactivation of viral genes, most likely prior to the presence of Tat as Vpr is packaged in the virion and is present upon infection (Hrimech et al., 1999). Vpr acts in a manner similar to that of ligand, binding GR and leading to the subsequent transactivation of viral genes through the GRE promoter region. The GR antagonist, mifepristone, is able to inhibit GR-mediated GRE transactivation. Here we have investigated mifepristone-mediated inhibition of virus replication in the context of HIV-1 infection using both transactivation assays as well as antiviral assays utilizing both CXCR4-using and CCR5-using viruses *in vitro*.

In order to first investigate the ability of mifepristone to inhibit the transactivation of the HIV-1 LTR promoter, we utilized reporter assays to determine if, in fact, mifepristone could inhibit transactivation. Mifepristone is known to inhibit GR-mediated transactivation in several models by competing for the binding sites with its ligand glucocorticoids (Kauppi et al., 2003; Pandit et al., 2002). Studies have shown that Vpr mimics glucocorticoids and that this activity is attained through its interaction with GR (Thotala et al., 2004) however it is unknown how structurally Vpr-GR-mifepristone functions. Future structure-modeling studies are required to identify the structural components involved in this interaction.

Next, we evaluated whether mifepristone could inhibit viral replication using the established HIV-1 target cell line CEM × 174. By utilizing the EGFP-expressing NL4-3 virus,

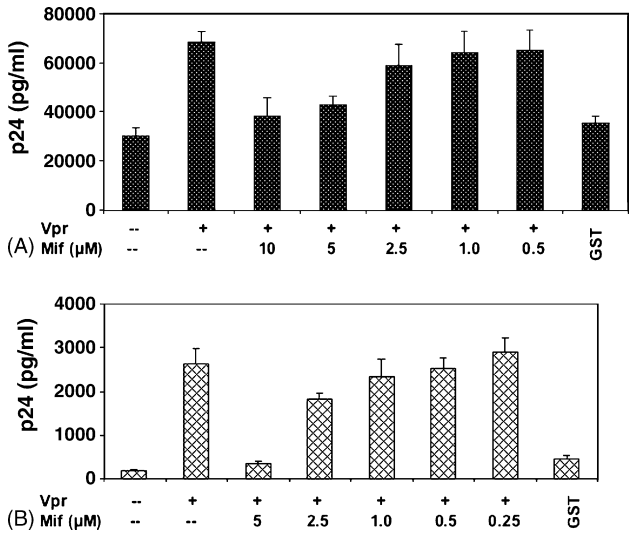

Fig. 6. Effects of mifepristone on virus reactivation in latently infected cells. Latently infected cell lines, OM-10.1 (Panel A) and U1 (Panel B) were activated with recombinant Vpr or GST (control) protein in the presence and absence of mifepristone. Virus replication was assayed on day 3 following activation by p24 released into the medium. Basal activity was assessed in the absence of Vpr and mifepristone (lane 1). Standard deviation (S.D.) was derived from three independent experiments.

we investigated the inhibition of virus replication in the presence of mifepristone by FACS analysis. Mifepristone inhibited virus replication significantly in infected cells following treatment. However, it is important to note that when mifepristone is removed from the culture the virus bounces back, indicating that mifepristone did not block the integration of the proviral DNA. Additionally, lower concentrations of mifepristone did not block virus replication suggesting that the effect of mifepristone could be limited in the absence of other antivirals targeting various stages of the virus life cycle.

To determine if mifepristone would be able to prevent replication in primary cells, we infected both PBMCs and monocyte-derived macrophages with dual tropic (CCR5 and CXCR4) (89.6) and CCR5-using (Ba-L) viruses, respectively, in the presence of mifepristone. Results demonstrated that similar to both transactivation models and the CEM cell model, mifepristone was able to inhibit viral replication in a dose-dependent manner. The importance in finding a compound that is able to prevent the re-emergence of infection in latent reservoirs prompted us to determine the effects of mifepristone in the latently expressing macrophage cell lines U1/HIV-1 and OM-10.1. Vpr is known to reactivate viral transcription in latent cells. Mifepristone was able to prevent reactivation by rVpr from a latent state in a dose-dependent manner, further demonstrating the ability of this compound to prevent reactivation of transcription in latent cell reservoirs.

Together these results demonstrate the effects of mifepristone both at the transcriptional level as well as in preventing both virus replication and reactivation from latency. While mifepristone is unable to fully prevent viral replication and the subsequent spread of replication-competent virus, it is possible that this compound could be used in conjunction with current HAART therapies to prevent replication and activation of HIV-1.

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