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Antireplicative and anticytopathic activities of prostratin, a non-tumor-promoting phorbol ester, against human immunodeficiency virus (HIV)[1]

Robert J. Gulakowski[a], James B. McMahon[a], Robert W. Buckheit Jr.[b], Kirk R. Gustafson[a], Michael R. Boyd[a,*]

[a] *Laboratory of Drug Discovery Research and Development, Developmental Therapeutics Program, Division of Cancer Treatment, Diagnosis and Centers, National Cancer Institute, NCI–Frederick Cancer Research and Development Center, Frederick, MD 21702-1201, USA*

[b] *Virology Research Division, Southern Research Institute–Frederick Research Center, Frederick, MD 21701, USA*

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Abstract

Prostratin, a non-tumor-promoting phorbol ester, inhibited human immunodeficiency virus (HIV)-induced cell killing and viral replication in a variety of acutely-infected cell systems. The potency and degree of cytoprotection was dependent on both viral strain and host cell type. Prostratin activated viral expression in two latently-infected cell lines, but had little or no effect on chronically-infected cell lines. Prostratin caused a dose-dependent, but reversible, decrease in CD4 expression in the CEM-SS and MT-2 cell lines. This down-regulation of CD4 was inhibited in a dose-dependent manner by the protein kinase C (PKC) antagonist, staurosporine. In addition, the cytoprotective and cytostatic effects of prostratin in CEM-SS cells acutely infected with HIV-1_{RF} were reversed by bryostatin-1, a PKC agonist. Prostratin had no effect on reverse transcriptase or HIV-1 protease, nor did it inhibit the binding of gp120 to CD4. We conclude that prostratin inhibits HIV cytopathicity and replication through mechanism(s) involving PKC enzyme(s). Copyright © 1997 Elsevier Science B.V.

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1. Introduction

A previous report described the bioassay-guided isolation and identification of a potent anti-human immunodeficiency virus (anti-HIV) constituent, prostratin, from extracts of the

* Corresponding author. Address: LDDRD, NCI-FCRDC, Bldg. 1052, Rm. 121, Frederick, MD 21702-1201. Fax: +1 301 8466177.

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Samoan medicinal plant *Homolanthus nutans* (Gustafson et al., 1992). The pure compound was initially shown to have activity against HIV-1_{RF} in three cell lines (CEM-SS, C-8166 and U937) and also in peripheral blood lymphocytes (PBL) and monocytes/macrophages (Gustafson et al., 1992). Structurally, prostratin is a member of the phorbol ester class, most members of which are known to be tumor promoters and protein kinase C (PKC) agonists. However, prostratin appears to represent a distinct subclass of PKC activator with unique biological activities that differ from tumor-promoting phorbol esters such as phorbol myristate–acetate (PMA) (Szallasi et al., 1992, 1993). Indeed, recent studies support the conclusion that prostratin is not a tumor promoter but is actually a potent anti-tumor promoter (Szallasi and Blumberg, 1991; Szallasi et al., 1992, 1993). For example, prostratin inhibited PMA induction of ornithine decarboxylase, edema and hyperplasia in CD-1 mouse skin (Szallasi and Blumberg, 1991); subsequent studies directly confirmed that prostratin inhibited tumor promotion by PMA (Szallasi et al., 1993). These strikingly atypical features of prostratin in comparison to other members of this class of compounds prompted us to further characterize its anti-HIV activity and potential mechanism(s) thereof.

2. Materials and methods

2.1. Cell lines and viruses

The lymphocytic cell lines, CEM-SS (Nara et al., 1987) and MT-2 (Harada et al., 1985) were maintained in RPMI 1640 medium (BioWhittaker, Walkersville, MD) without phenol red and supplemented with 10% fetal bovine serum (FBS) (BioWhittaker), 2 mM L-glutamine and 50 μg/ml gentamicin (BioWhittaker) (complete medium). ACH-2 cells latently infected with HIV-1_{LAV} (Folks et al., 1989), and U1 cells latently infected with HIV-1 (Folks et al., 1987), were maintained in complete medium supplemented with 10% FBS. Exponentially growing cells were pelleted and resuspended in complete medium at the desired density. For studies involving acute infections, HIV-1_{RF}, HIV-1_{IIIB} and HIV-2_{ROD} were used to infect CEM-SS or MT-2 cells. Frozen virus stock solutions were thawed immediately before use and resuspended in complete medium to yield the desired multiplicity of infection (MOI) (80–90% cell killing). Infections were allowed to proceed for 4–6 days at 37°C in a humidified atmosphere containing 5% CO_2. Anti-HIV activity was assessed by quantitating cellular viability using the metabolic reduction of the tetrazolium salt, XTT and/or by monitoring viral replication by measuring supernatant reverse transcriptase (RT) activity, p24 antigen production and/or synthesis of infectious virions as previously described (Gulakowski et al., 1991).

2.2. Compounds

Prostratin (95% pure) was obtained from LC Laboratories (Woburn, MA) and was purified to homogeneity by trituration with toluene. After washing twice, the toluene-insoluble material was resuspended in CH_2Cl_2. The purity of the prostratin was determined by nuclear magnetic resonance (NMR) analysis. Bryostatin-1 and 3′-azido-3′-deoxythymidine (AZT) were obtained from the Drug Synthesis and Chemistry Branch, NCI, NIH. Staurosporine was purchased from Sigma (St. Louis, MO).

2.3. Effects of prostratin on latently-infected cells

ACH-2 cells and U1 cells were used to determine the effect of prostratin on virus replication as measured by the accumulation of supernatant RT activity. Briefly, 4×10^4 cells were added in 100-μl aliquots to individual wells of a 96-well microtiter plate containing 100 μl of prostratin or medium alone. Plates were incubated for 24–96 h at 37°C. Following incubation, supernatant fluids were harvested, centrifuged to remove any residual cells and tested for RT activity as previously described (Buckheit and Swanstrom, 1991).

2.4. Pretreatment of HIV-1_{RF} with prostratin

Concentrated HIV-1_{RF} was pretreated for 1 h with 1 μM prostratin or medium alone. After

incubation, the pretreated virus supernatant was diluted to yield a MOI of 0.8 and to dilute the prostratin beyond an effective antiviral concentration. The prostratin-treated virus (50 μl) was then added to individual wells of a 96-well microtiter plate containing 5000 CEM-SS cells (50 μl) and either 100 μl of medium alone or 1 μM prostratin. Plates were incubated for 6 days and cellular viability was assessed using the XTT assay.

2.5. Pretreatment of CEM-SS cells with prostratin

CEM-SS cells were incubated with 25 μM prostratin or complete medium for 1 h at 37°C. After incubation, CEM-SS cells were washed free of prostratin using two centrifugation steps. Pretreated cells were then resuspended in complete medium and added to individual wells of a 96-well microtiter plate (5000 cells/50 μl) containing 100 μl of medium alone or 1 μM prostratin. A 50 μl aliquot of diluted HIV-1_{RF} was added to appropriate wells to yield a MOI of 0.8. Plates were incubated for 6 days at 37°C in an atmosphere containing 5% CO_2. After incubation, cellular viability was assessed using the XTT assay.

2.6. Delayed addition of prostratin to HIV-infected cells

CEM-SS cells were plated into individual wells of a 96-well microtiter plate at a density of 5000 cells/well in 50 μl of medium. Diluted HIV-1_{RF} stock supernatants (50 μl) were added to appropriate wells to yield a MOI of 1.0. At various times after the addition of virus, a 100-μl aliquot of 20 μM prostratin or 1 μM AZT was added to appropriate wells. After 6 days of incubation, cellular viability was assessed using the XTT assay and viral replication was assessed by measuring the RT activity in prostratin-treated, HIV-1-infected culture supernatants.

2.7. RT assay

Prostratin was assayed for its inhibitory activity against recombinant HIV-1 (Hizi et al., 1988) and HIV-2 (Hizi et al., 1991) RT as previously described (Kashman et al., 1992).

2.8. Effect of prostratin on CD4 expression

CEM-SS and MT-2 cells were pretreated for 4 or 24 h with 0.01–10 μM prostratin, pelleted, stained for 15 min with a fluorescein isothiocyanate (FITC)-labeled anti-Leu-3a at 4°C, washed twice with phosphate buffered saline (PBS) and analyzed by flow cytometry using a FACScan machine (Becton Dickinson, San Jose, CA). Cells were stained with a FITC-labeled IgG_1 antibody as a control. In an additional experiment, CEM-SS cells were incubated for 30 min with various concentrations of staurosporine (1–100 nM), followed by an additional 4-h incubation with 10 μM prostratin. The CEM-SS cells were then analyzed for CD4 expression as before.

2.9. Protease activity

Prostratin (25 μM) was tested in a high-performance liquid chromatography (HPLC)-based HIV-1 protease inhibition assay as previously described (Louis et al., 1989).

2.10. gp120/CD4 binding assay

The effect of prostratin on the binding of gp120 to CD4 was analyzed using an antigen capture enzyme-linked immunosorbent assay (ELISA) (DuPont, Wilmington, DE) according to the manufacturer's directions. Optical density (OD) was read using a V_{max} microplate reader (Molecular Devices, Menlo Park, CA).

2.11. Effect of bryostatin-1 on the anti-HIV activity of prostratin

CEM-SS cells (5000 cells/50 μl) were added to individual wells of a 96-well microtiter plate containing 100 μl of either prostratin, bryostatin-1 or a combination of the two. Diluted HIV-1_{RF} stocks (50 μl) were added to appropriate wells to yield a MOI of 0.8. Plates were incubated for 6 days. Cellular viability was assessed using the XTT assay and viral replication was assessed by measuring RT activity in culture supernatants.

3. Results

3.1. Effect of prostratin on acute infections

Prostratin was assayed for anticytopathic activity against different strains of HIV in the CEM-SS (Fig. 1(A–C)) and MT-2 (Fig. 1(D–F)) cell lines. Prostratin protected CEM-SS cells but not MT-2 cells from the cytopathic effects of HIV-1_{RF} (Fig. 1(A) and (D)) and HIV-1_{IIIB} (Fig. 1(B) and (E)) at concentrations of 0.3–50 μM. These concentrations were also cytostatic and therefore resulted in low OD readings for both infected and uninfected cells. In addition, prostratin failed to inhibit replication of HIV-1_{RF} in the C-344 and LDV-7 cell lines (data not shown). Interestingly, prostratin inhibited replication of HIV-2_{ROD} in both the CEM-SS (Fig. 1(C)) and MT-2 (Fig. 1(F)) cell lines at concentrations of 0.05–50 μM and 0.2–50 μM, respectively, which were also cytostatic. Previous studies (Gustafson et al., 1992) have shown that prostratin was very effective at inhibiting viral replication at cytoprotective, although also cytostatic, concentrations (0.5–50 μM). However, at sub-protective concentrations of prostratin (e.g. 0.01–0.1 μM) there were markedly increased supernatant accumulations of viral particles or products, including RT, p24 antigen and infectious virions (Gustafson et al., 1992). In an attempt to ascertain whether this represented an actual enhancement by prostratin of the rate of viral replication after acute infection, or rather if it simply represented a prolongation of the ability of the lethally infected cells to support viral replication, supernatants from prostratin-treated, HIV-1_{RF}-infected cultures were harvested after 4, 5 or 6 days of incubation and analyzed for net viral replication as previously described (Gulakowski et al., 1991). Supernatants harvested on day 4 showed viral replication indices essentially equivalent to viral controls (Fig. 2(A)). Results from supernatants harvested on day 5 revealed (Fig. 2(B)) an intermediate 2.5-fold increase in viral indices compared with control levels, whereas the day 6 analysis revealed a 4.5-fold increase (Fig. 2(C)).

3.2. Effect of prostratin on established infections

Latently-infected ACH-2 or U1 cells incubated with prostratin for 48 h resulted in a substantial activation of virus production as measured by supernatant RT activity (Table 1).

3.3. Pretreatment of HIV-1_{RF} or CEM-SS cells with prostratin

To determine if prostratin exerted a direct virucidal effect, concentrated HIV-1_{RF} was pretreated with 1 μM prostratin for 1 h. After incubation, the solution was diluted below a normally effective concentration of prostratin to yield an appropriate MOI after addition of the CEM-SS target cells. The pretreated virus remained highly lethal to the cells and caused approximately 85% cell death, similar to the virus control. Maximal cytoprotection could be recovered only by re-introduction and the continuous presence of prostratin (data not shown). Similarly, the possibility that prostratin might irreversibly bind or block cellular receptors for HIV, or that it might accumulate or act otherwise intracellularly to prevent subsequent susceptibility to HIV infection, was evaluated. CEM-SS cells were pretreated with 25 μM prostratin for 1 h or up to 4 days (data not shown), followed by centrifugation and removal of the prostratin-containing medium, followed by introduction of the virus. The prostratin-pretreated cells remained highly susceptible to infection and lethality by HIV (data not shown); maximal cytoprotection could be recovered only by re-introduction and continuous maintenance of an effective concentration of prostratin.

3.4. Delayed addition of prostratin to infected cells

Time-course studies involving the addition of prostratin at various times after the initiation of HIV infection were undertaken (Fig. 3). Results from the XTT viability assay revealed that prostratin was still fully cytoprotective when added as late as 30 h after infection. Addition of prostratin 48 h or longer after infection resulted in markedly diminished or no cytoprotection. In contrast to

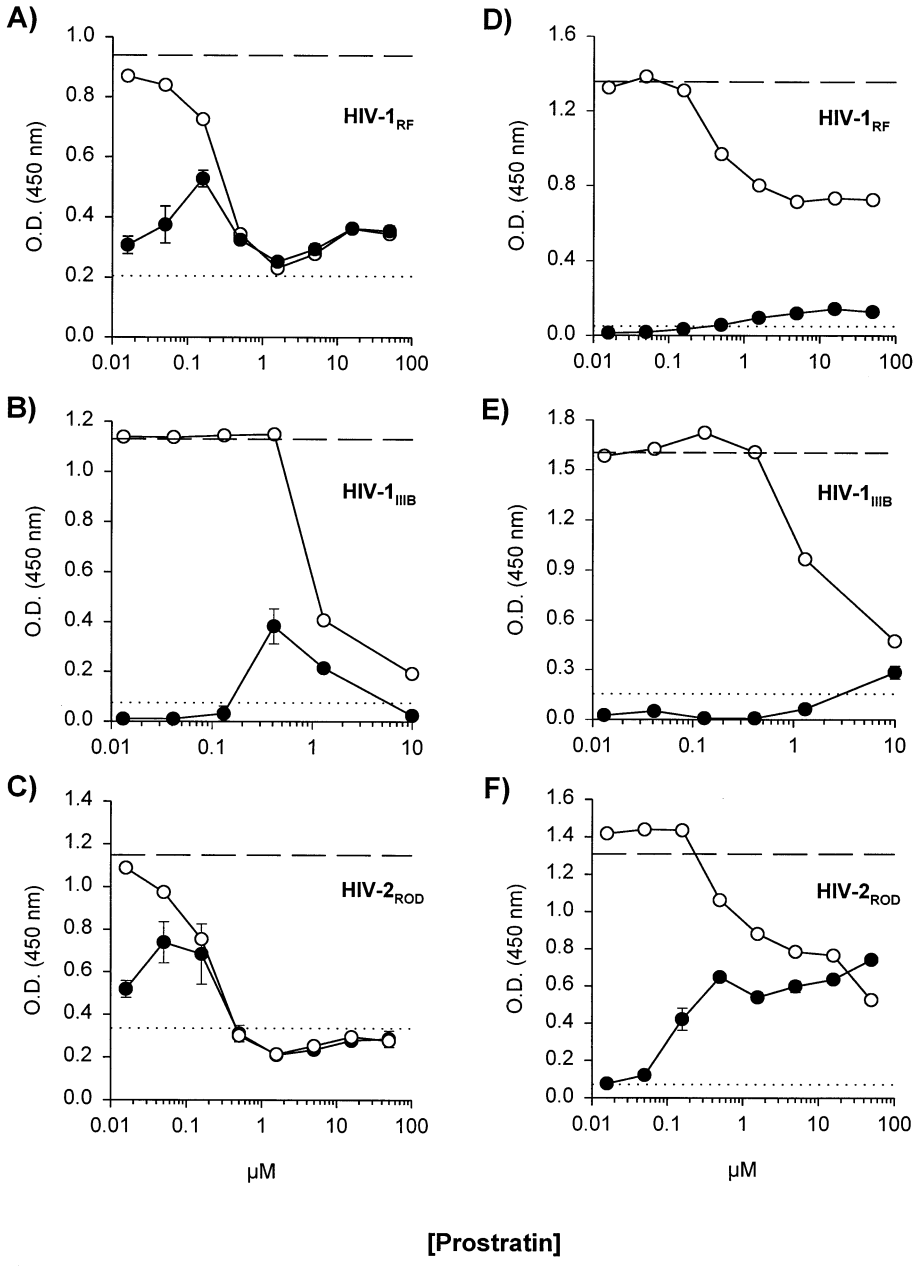

Fig. 1. Anti-HIV activity of serial dilutions of prostratin against acute HIV-1_{RF}, HIV-1_{IIIB} and HIV-2_{ROD} infections in the CEM-SS (A–C) or MT-2 (D–F) cell lines. Cellular viability was assessed after 6 days in culture using the XTT assay (Gulakowski et al., 1991). Results are graphically displayed as the mean optical density (OD) values ± S.D. for infected, prostratin-treated (●) and uninfected, prostratin-treated cells (○). Mean OD values obtained for untreated, infected controls (·····) and untreated, uninfected controls (—— ——) are also shown.

the cytoprotective effects, maximal suppression of viral replication required an essentially immediate addition of prostratin. Delaying the addition of prostratin as little as 4 h resulted in a significant level of supernatant RT activity (Fig. 3). Delaying the addition of AZT to infected cultures more

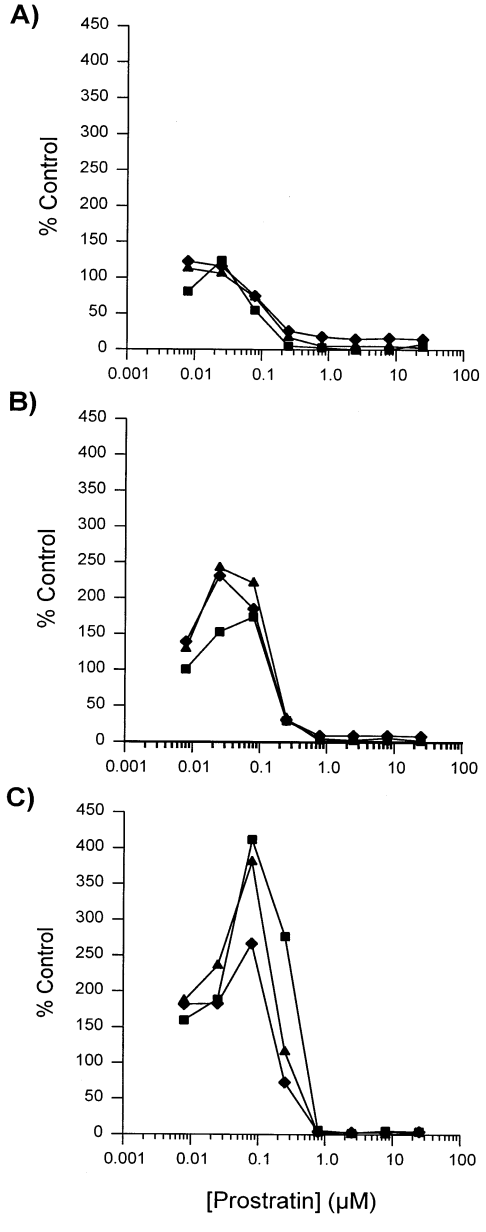

Fig. 2. Comparison of supernatant accumulations of viral replication indices of prostratin-treated, HIV-1-infected CEM-SS cell cultures after (A) 4, (B) 5 and (C) 6 days in culture. Supernatants were assayed for RT activity (▲), p24 antigen production (◆) and the synthesis of infectious virions (■) as described (Gulakowski et al., 1991). The results are graphically represented as % of the untreated, infected controls (S.E.M. ⩽ 15%). Control values for viral replication indices on days 4, 5 and 6 were as follows. RT activity (cpm): 1701, 1978, 5617; p24 antigen (μg/ml): 12, 92, 114; infectious virions (SFU/ml): 1.9×10^5, 2.2×10^5, 2.1×10^5.

than 6 h caused a significant decrease in the antiviral activity of the nucleoside analog (Fig. 3).

3.5. Down-regulation of CD4 expression by prostratin

CEM-SS and MT-2 cells were treated with varying concentrations of prostratin for 4 or 24 h, pelleted, stained with a FITC-labeled anti-Leu-3a for 15 min and then subjected to FACScan analysis. Prostratin caused a dose-dependent decrease in CD4 expression in both the CEM-SS (Fig. 4(A)) and the MT-2 (Fig. 4(B)) cell lines as indicated by a decrease in fluorescence intensity compared with medium-treated control cells after 4 h (Fig. 4(A–B)) and 24 h (data not shown). Removal of prostratin resulted in a return to control levels of CD4 expression after 12 h. To determine the effect of staurosporine, a known PKC inhibitor (Boto et al., 1991) on the down-regulation of CD4 by prostratin, CEM-SS cells were treated with various concentrations of staurosporine for 30 min, then additionally with 10 μM prostratin for 4 h. Staurosporine inhibited the down-regulation of CD4 by prostratin in a dose-dependent manner, resulting in a greater level of CD4 expression compared with cells treated with 10 μM prostratin alone (Fig. 4(C)). Staurosporine alone caused an 18% decrease in CD4 expression at the top dose of 100 nM.

3.6. Effect of bryostatin-1 on the anti-HIV activity of prostratin

Bryostatin-1 (Blumberg and Pettit, 1992) reversed the ability of prostratin to inhibit the cytopathic effect of HIV-1_{RF} in a dose-dependent manner (Fig. 5). This resulted in an increase in syncytial formation (visible observations) and an increase in supernatant RT activity compared with prostratin-treated control cultures. In addition, lower OD readings of infected cultures were observed with increasing concentrations of bryostatin-1 compared with their uninfected counterparts. Bryostatin-1 also reversed the cy-

Table 1
Effects of prostratin on viral replication in latently infected cells

Cell line	Conc. (μM)	cpm $\pm$ S.D. (% control)
U1	0	866 $\pm$ 103
	0.10	1289 $\pm$ 71 (149)
	0.32	4019 $\pm$ 757 (464)
	1.0	4185 $\pm$ 1177 (483)
ACH-2	0	4661 $\pm$ 1477
	0.1	33 186 $\pm$ 2964 (112)
	0.32	88 966 $\pm$ 10976 (1909)
	1.0	47 738 $\pm$ 6099 (1024)

Effects were estimated by the supernatant RT activities after 48 h of incubation with 0.1, 0.32 or 1 μm prostratin. Mean values and standard deviations were calculated from triplicate determinations and displayed as cpm $\pm$ S.D.; % of untreated controls are displayed in parenthesis.

tostatic effect of prostratin as evidenced by an increase in OD values in uninfected CEM-SS cells compared with uninfected cells treated with prostratin alone. Bryostatin-1 alone had no anti-HIV or cytostatic activity in these assays (data not shown).

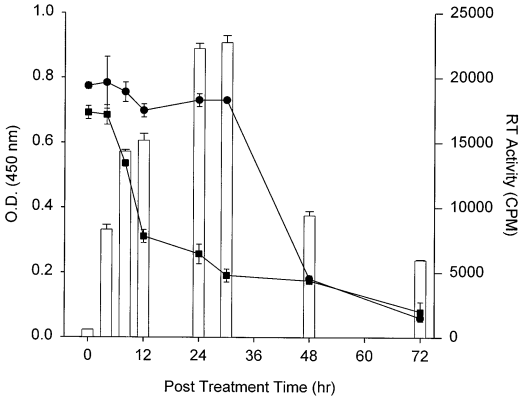

Fig. 3. Effect of a delayed addition of prostratin (●) or AZT (■) to infected cultures. Prostratin or AZT was added to infected CEM-SS cells at various times after the initiation of the infection (0, 4, 8, 12, 24, 30, 48 and 72 h). Following a 6-day incubation, cellular viability was assessed using the XTT assay. Results are graphically displayed as the mean OD $\pm$ S.D.. OD values for the virus and cell controls were 0.09 and 0.7, respectively. Viral replication was monitored by measuring supernatant levels of RT (□) in prostratin-treated infected cultures. Results are displayed as the cpm $\pm$ S.D. of triplicate determinations. The cpm obtained for infected controls was 7587 $\pm$ 418.

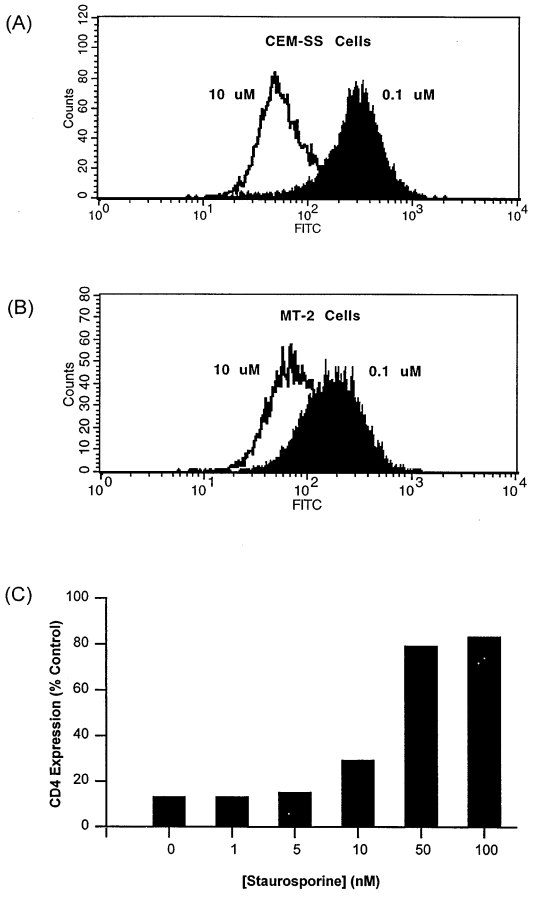

Fig. 4. Down-regulation of CD4 expression by prostratin. FACScan analysis of CD4 expression of CEM-SS (A) or MT-2 (B) cells stained with a FITC-labelled anti-Leu-3a antibody after exposure to 0.1, 1.0 (data not shown), or 10 μM prostratin for 4 h. Data are expressed as the number of cells (counts) vs. the intensity of fluorescence (FITC) obtained for each condition. Cells were also stained with a FITC-labelled IgG_1 as a control. Mean channel fluorescence for the IgG_1 control was 6.27. (C) FACScan analysis of CD4 expression of CEM-SS cells stained with a FITC-labelled anti-Leu-3a antibody after exposure to 1, 5, 10, 50 or 100 nM staurosporine for 30 min followed by a 4-h co-incubation with 10 μM prostratin. Data are expressed as the mean channel fluorescence of the prostratin and staurosporine-treated cells compared with the medium-treated control. Staurosporine (100 nM) caused an 18% reduction in CD4 expression compared with the medium control (data not shown).

3.7. Biochemical assays

Prostratin failed to inhibit either HIV-1 or HIV-2 recombinant RT in enzyme inhibition as-

says at concentrations of up to 25 μM (data not shown). Likewise, prostratin (25 μM) had no effect on the activity of a recombinant HIV-1 protease (data not shown). In addition, prostratin failed to inhibit the binding of gp120 to CD4 in an antigen capture ELISA at concentrations up to 50 μM (data not shown).

4. Discussion

Empirical screening of extracts from the Samoan medicinal plant *Homalanthus nutans* led to the initial discovery of the anti-HIV activity of prostratin, a non-tumor-promoting phorbol ester (Gustafson et al., 1992). Prostratin was interesting in that it bound to and activated PKC; however, in mouse skin it either failed to induce the typical phorbol ester responses (e.g. hyperplasia) or induced only a partial response (e.g. inflammation) (Gustafson et al., 1992). In addition, pretreatment with prostratin inhibited a range of PMA-induced effects, including tumor promotion (Szallasi and Blumberg, 1991; Szallasi et al., 1993). These unusual features of prostratin spurred our interest in further investigating its anti-HIV properties.

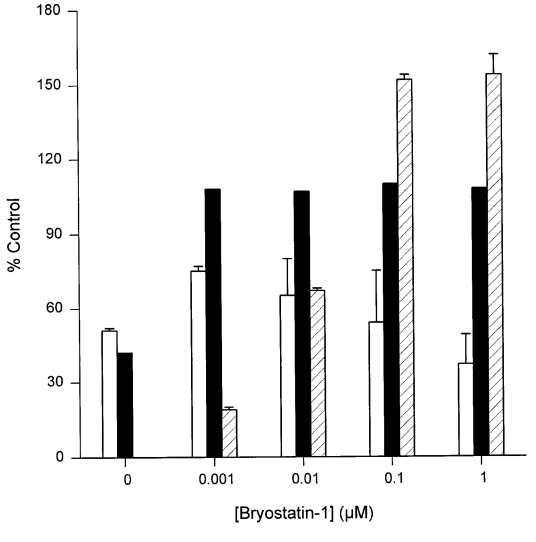

Fig. 5. Effects of bryostatin-1 on the antiviral activity of prostratin. HIV-1-infected (□) and uninfected (■) CEM-SS cells were incubated in the presence of 25 μM prostratin and/or bryostatin-1 (0.001, 0.01, 0.1 or 1.0 μM) for 6 days. Cellular viability was assessed using the XTT assay. Results are graphically displayed as % of the uninfected, untreated control OD value (1.1) $\pm$ S.D.. Supernatant levels of RT (▩) were also measured. Results are displayed as % of the untreated, infected cultures (cpm = 6199).

The initial antiviral studies (Gustafson et al., 1992) revealed that prostratin strongly inhibited HIV-1 in CEM-SS, C-8166 and U937 cells as well as in PBLs and macrophages. To further define the biological range of activity of prostratin, we have tested its anti-HIV activity in a number of different host cell/virus strain combinations, and have found that the activity is dependent upon both the particular host cell line and virus strain. For example, the compound protected CEM-SS (Fig. 1(A)) and C-8166 from the cytopathic effects of HIV-1_{RF} but failed to protect MT-2 (Fig. 1(D)), LDV-7, or C-344 cells (data not shown) from acute infection with the same strain of HIV-1. Similar results were found with the IIIb strain of HIV-1, in that prostratin inhibited replication in the CEM-SS (Fig. 1(B)) but not the MT-2 (Fig. 1(E)) cell line. Interestingly, prostratin inhibited replication of HIV-2_{ROD} in both the CEM-SS (Fig. 1(C)) and the MT-2 (Fig. 1(F)) cell lines.

In CEM-SS cells acutely infected with HIV-1_{RF}, prostratin completely inhibited viral cytopathicity and replication; however, only at concentrations which were also strongly (but reversibly) cytostatic (Gustafson et al., 1992). Cultures containing sub-cytoprotective concentrations of prostratin consistently showed greater supernatant accumulations of viral replication indices than the corresponding infected, untreated controls (Gustafson et al., 1992). It was therefore important to determine whether this was due to an actual stimulation of the rate of viral replication by prostratin or whether it simply reflected a net greater accumulation over time of viral particles from cells which, in the presence of prostratin, were being kept alive and producing virus longer than their untreated counterparts. To accomplish this we compared supernatants harvested from prostratin-treated, HIV-infected cells after 4, 5 and the standard 6 days of incubation. Analysis of the day 4 supernatants (Fig. 2(A)) revealed no difference in the viral indices (RT, p24 and infectious virions)

compared with the corresponding untreated controls. However, day 5 supernatants (Fig. 2(B)) revealed a 2.5-fold increase and day 6 supernatants (Fig. 2(C)) showed a 4.5-fold increase. This result was consistent with the view that the apparent 'spike' in supernatant replication indices observed with the longer incubation times reflected a net greater accumulation of viral particles, due to prolonged host cell survival rather than a direct stimulation or enhancement of the rate of viral replication.

In contrast to its inhibitory effect on acute HIV infection, prostratin had different effects on several chronically (i.e. actively producing, but not killed by the virus) or latently infected (i.e. containing the provirus but producing little or no detectable HIV) T-cell lines. Prostratin did not diminish nor stimulate virus production in several cell lines chronically-infected with HIV-1 (e.g. H9/HIV-$1_{IIIB,}$ H9/HIV-1_{SK1}, CEM/HIV-1_{SK1}, U937/HIV-1_{SK1}) (data not shown). However in two latently infected cell lines, ACH-2 and U1, prostratin significantly activated virus production, as measured by supernatant RT activity (Table 1). These results are comparable to results obtained with PMA (Folks et al., 1989).

It is well known that phorbol esters modulate the expression of CD4 on the surface of T-lymphocytes (Acres et al., 1986; Hoxie et al., 1988; Golding et al., 1994). We therefore analyzed the effect of prostratin on CD4 expression in both the CEM-SS and the MT-2 cell lines. Prostratin caused a dose-dependent decrease in CD4 expression in both the CEM-SS (Fig. 4(A)) and the MT-2 (Fig. 4(B)) cell lines after 4 h of incubation. Identical results were obtained for both cell lines after 24 h of incubation with prostratin (data not shown). The modulation of CD4 by prostratin was completely reversible by 12 h after the elimination of the compound; the modulation could also be reversed by the presence of the PKC inhibitor, staurosporine (Fig. 4(C)). The anticytopathic activity of prostratin is most likely not related to its modulation of CD4 expression, since prostratin failed to protect MT-2 cells from the lethal effects of infection with HIV-1_{RF} but caused a decrease in CD4 expression similar to that found in the CEM-SS cells.

The possible involvement of PKC in the antiviral activity of prostratin was examined using the known PKC agonist, bryostatin-1. Bryostatin-1 reversed both the cytoprotective and the cytostatic effects of prostratin in a dose-dependent manner (Fig. 5) in the 6-day acute infection assay, resulting in an increase in cytopathicity and viral replication in infected cultures and an increase in cell growth in uninfected cultures. This effect of bryostatin on prostratin is also consistent with that reported for bryostatin-1 on PMA in the Jurkat T-cell line (Levine et al., 1991), and likewise in human breast cancer cell lines where the effect of bryostatin-1 is dominant over that of PMA (Kennedy et al., 1992).

In summary, prostratin has shown an anti-HIV activity pattern similar to other phorbol esters; however, it differs markedly in its lack of known tumor-promoting properties (Gustafson et al., 1992). The compound's anti-HIV activities in acutely, chronically and latently infected cells is interestingly dependent upon the particular virus strains and host cells employed in the assays. In the CEM-SS/HIV-1_{RF} assay system, prostratin had no direct effects on the virus (data not shown) or host cells (data not shown) and required continuous presence for its anticytopathic activity. Time-course studies revealed that prostratin could be added as late as 30 h post-infection and still provide protection from the cytopathic effects of HIV (Fig. 3). Delaying the addition of AZT for more than 6 h caused a significant loss of cytoprotection. In contrast, viral replication was unaffected if prostratin was added as little as 4 h after the infection was initiated. Biochemical studies revealed that prostratin did not inhibit the activity of a recombinant HIV-1 RT or HIV-2 RT over a broad range of concentrations nor did it inhibit the activity of a recombinant HIV-1 protease. In addition, prostratin failed to block the binding of gp120 to soluble CD4 in an antigen capture ELISA.

Mechanistically, the anti-HIV activity of prostratin appears most closely correlated with its expected modulatory effects on PKC enzyme(s), since treatment with bryostatin-1 (a PKC agonist) or staurosporine (a PKC antagonist) reversed the effects of prostratin in the CEM-SS cell line. The

general lack of effective chemotherapeutic agents for the treatment of AIDS warrants a closer examination of any compound with potentially exploitable anti-HIV activity. Rational combinations of drugs active at different stages in the life cycle of HIV and/or pathway(s) of HIV-induced cell death will likely be required for effective chemotherapy of HIV-infection and AIDS. In this respect, the ability of a non-tumor-promoting phorbol, such as prostratin, to inhibit new infection and/or cell killing of cells by HIV, while at the same time stimulating latently infected cells to express HIV, might provide a basis to explore an unconventional therapeutic strategy. For example, the synchronous activation of latently infected cells by prostratin, simultaneously in the presence of a reagent (e.g. an immunotoxin) capable of recognizing and killing HIV-expressing cells, and perhaps additionally in the presence of another type(s) of agent to further inhibit viral infectivity and/or cell killing, might provide a means to eliminate a critical and otherwise inaccessible reservoir of cells silently harboring provirus.

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