DIFFERENT EFFECTS OF PHORBOL ESTER DERIVATES ON HUMAN IMMUNODEFICIENCY VIRUS 1 REPLICATION IN LYMPHOCYTIC AND MONOCYTIC HUMAN CELLS

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Summary. – The mode of action of the phorbol 12,13-dibutyrate (PDBu) and phorbol 12-myristate 13-acetate (PMA) on the human immunodeficiency virus 1 (HIV-1) replication in human lymphocytes and monocytes was studied. PDBu and PMA appear to have similar effects on the regulation of HIV-1 replication in acutely infected cells. Here we show a significantly increased replication of HIV-1 induced by PDBu and PMA in Molt-4 and Jurkat cells, but a reduced replication in THP-1 and U-937 cells. Moreover, quantitatively different activity of the two derivatives in relation to HIV-1 replication was observed. PDBu proved to be a stronger stimulator or suppressor of HIV-1 replication as compared to PMA. Although the precise mechanism of the activation of HIV-1 replication by phorbol ester derivatives is not clear, it can be assumed that the hydrophilycity of PDBu may cause its stronger effect.

Introduction

Gene expression of HIV-1 can be induced by different signals including cellular factors and HIV-1 gene products (Bednarik and Folks, 1992). Different exogenous factors can also induce the expression of HIV-1, including mitogens, antigens, heterologous viral transactivators, UV-irradiation or phorbol ester derivatives (Rosenberg and Fauci, 1989).

Phorbol esters have pleiotropic effects on cells, such as changes of their morphology, membrane transport, cyclic nucleotide synthesis, stimulation of mitosis, and induction of enzymes (Weinstein *et al.,* 1979). They rapidly enter the cells and once being in the cytoplasm they behave as diacylglycerol, an endogenous intracellular messenger, and permanently activate the protein kinase C (PKC) system (Castagna *et al.,* 1982).

A representative phorbol ester studied extensively with respect to the regulation of HIV-1 replication trough stimulation of PKC is a lipophylic compound PMA (12-O-tetradecanoylphorbol-13-acetate, TPA). It has been shown that PMA enhances HIV-1 replication in T cells by inducing a cellular factor NF-kB which binds to the enhancer region of HIV-1 LTR (Gaynor, 1992), acts through PKC and inhibits HIV-1-induced syncytia formation but enhances HIV-1 production in Molt-4 cells (Chowdhury *et al.,* 1990). Little is known, however, about the PMA effects on HIV-1-infected monocytic cells. Moreover, no information about the possible effect of hydrophilic phorbol ester derivates, e.g. PDBu, on HIV-1 replication is available.

The aim of the present study was to investigate and to compare the action of two different phorbol ester derivatives on HIV-1 replication in cells of T-lymphocyte and monocyte lineages in acute infection.

Materials and Methods

Phorbol derivatives. PDBu ($C_{36}H_{40}O_8$, FW 504.6) and PMA ($C_{36}H_{56}O_8$, FW 616.8) (Sigma) were stored at -30°C as 1 mmol/l stock solutions in dimethyl sulfoxide and RPMI-1640, respectively, until use.

Cells. Human lymphocytic cell lines Jurkat and Molt-4 clone 8 (Walder *et al.,* 1995), and monocytic cell lines U-937 and THP-1

Abbreviations: CPE = cytopathic effect; FCS = foetal calf serum; HIV-1 = human immunodeficiency virus 1; PDBu = phorbol 12, 13-dibutyrate; p.i. = post infection; PKC = protein kinase C; PMA = phorbol 12-myristate 13-acetate; RT = reverse transcriptase; TCA = trichloracetic acid
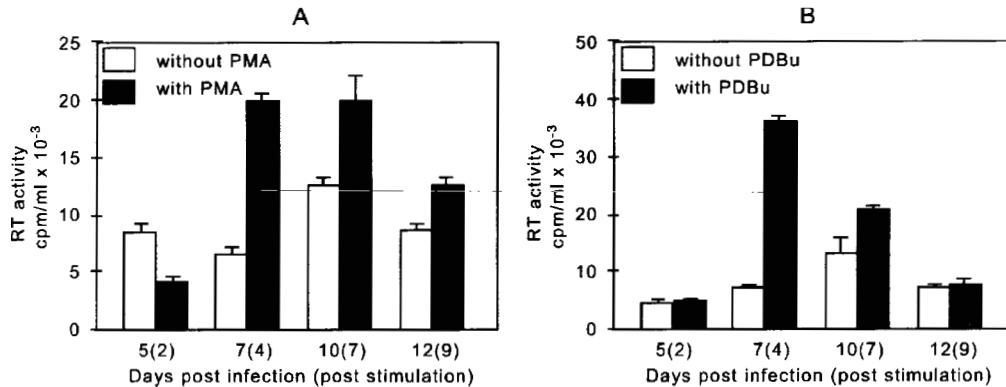
Fig. 1
Stimulation of HIV-1 replication in lymphocytic Molt-4 cells with PMA (A) and PDBu (B)
Values are given as means ± SD of six values (results of 2 experiments run in triplicate).

(Tsuchiya *et al.,* 1980) were maintained in RPMI-1640 medium supplemented with 10% foetal calf serum (FCS). 2000 U/ml penicillin, 125 µg/ml streptomycin and 5 µg/ml fungizone.

Virus. The HIV-1 IIIB strain (Popovic *et al.,* 1984), used throughout this study, was propagated in Jurkat cell line. Stock virus had a reverse transcriptase (RT) activity of 1.6×10^6 cpm/ml and an infectivity titer of $10^{4.5}$ $TCID_{50}$/ml.

Induction of HIV-1 replication in cells by PDBu and PMA. Each type of cells was infected with HIV-1 at a multiplicity of infection (MOI) of 0.01 $TCID_{50}$ per cell and incubated for 1 hr at 37°C in suspension. Then, tissue culture multi-well plates FB-24 (Linbro, Flow Laboratories) were seeded with 2×10^5 cells per well in 2 ml of RPMI medium supplemented with 10% FCS and antibiotics, and incubated at 37°C in 5% CO_2. All experiments were carried out in triplicate. Seventy-two hrs post infection (p.i.), samples were taken for RT assay and 1 ml of the medium with 10^{-6} mol/l PMA or 3×10^{-7} mol/l PDBu was added, respectively. Higher concentrations of PMA and PDBu were found to cause a reduced cell viability and growth (data not shown). The concentrations of PMA and PDBu used currently in our experiments were sufficient to induce optimal effects on HIV-1 replication and permited a normal cell growth. Every 48 hrs post induction the number of viable cells in each culture was counted in a standard way using the trypan blue exclusion method (Merchant *et al.,* 1960) and RT in supernatant was assayed. As controls, cells incubated with plain medium only were included in each experiment. Morphological changes and virus-mediated cytopathic effect (CPE, syncytium formation) were observed daily.

RT assay was performed according to Kalvatchev *et al.* (1995) with modifications. The tissue culture supernatant was harvested and precipitated with 30% polyethylene glycol (MW 8,000) in 1 mol/l NaCl. The virions were pelleted by centrifugation at 5,000 rpm for 20 mins and disrupted with a solubilization buffer containing 50 mmol/l Tris.HCl pH 7.8, 0.8 mol/l NaCl, 0.5% Triton X-100, 0.5 mmol/l phenylmethyl-sulfonyl fluoride and 20% glycerol. Twenty µl of this sample was mixed with 180 µl of the reaction mixture containing 50 mmol/l Tris.HCl pH 7.8, 10 mmol/l $MgCl_2$, 5 mmol/l dithiothreitol,

0.02 units (A_{260}) of poly (rA):poly $(dT)_{12-18}$ (template-primer, Pharmacia), 0.16 mol/l dATP and 52 uCi/ml [methyl-^{3}H]TTP (10-25 Ci/mmol), and incubated at 37°C for 2 hrs. Then 3 ml of 10% trichloroacetic acid (TCA) containing 0.02 mol/l sodium pyrophosphate was added. After 20 mins at 4°C the precipitated DNA was collected on GN-4 (Gelman) filter and washed with 10% TCA. The filter was dried under a heat lamp and placed into 5 ml of Aquasol-2 (NEN, DuPont) for the ^{3}H-radioactivity counting in a Packard liquid scintillation spectrometer. A supernatant from non-infected cell culture and a concentrated HIV-1 served as negative and positive controls, respectively. The RT activity was expressed in cpm/ml.

Results

Stimulation of HIV-1 replication in acutely infected lymphocytic cells with PMA and PDBu

To examine the effect of the phorbol esters on HIV-1 replication in Molt-4 and Jurkat cells, levels of RT activity were measured over 9 days after induction with PMA or PDBu. To better understand the kinetics of HIV replication in these cells after PMA and PDBu induction, supernatants were harvested every 48 hrs over 9 days. We observed that the steady-state level of RT activity in Molt-4 clone 8 cell increased within 4 days post treatment about three-fold and five-fold after treatment with PMA (Fig. 1A) and PDBu (Fig. 1B), respectively, as compared to untreated controls. Similar results were found with Jurkat cells (data not shown).

Reduction of HIV-1 replication in acutely infected monocytic cells with PMA and PDBu

PMA and PDBu induced a dramatic suppression of HIV-1 replication during 9 days post the treatment in THP-1 (Fig.
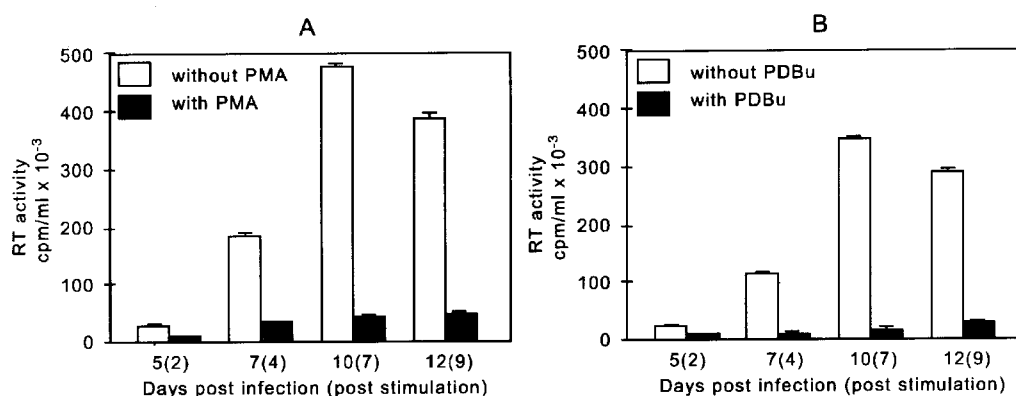
Fig. 2
Inhibition of HIV-1 replication in monocytic THP-1 cells with PMA (A) and PDBu (B)
Values are given as means ± SD of six values (results of 2 experiments run in triplicate).

2) and U-937 cells (data not shown) as compared to controls. Interestingly, in the absence of either PMA or PDBu treatment, RT activity was detected 5 days p.i. and reached its highest level at 10 days p.i. (Fig. 2). In contrast, when the cells were treated with PMA or PDBu, only low levels of RT activity were detected until 12 days p.i.. At the peak of RT activity in untreated THP-1 cells (10 days p.i.), the RT values were about 12-fold and 28-fold lower in the cells treated with PMA (Fig. 2A) and PDBu (Fig. 2B), respectively. In addition, after 2 hrs of treatment with PMA or PDBu, the adherence of THP-1 and U-937 cells increased from less than 1% to more than 90% while the cell viability remained unchanged.

Different effect of PDBu and PMA on HIV-1 replication in lymphocytic and monocytic cells

A comparison of the effects of PMA and PDBu on HIV-1 replication in lymphocytic (Molt-4) and monocytic (THP-1) cells showed (Fig. 3) that both the stimulatory and inhibitory effects of PDBu on Molt-4 and THP-1 cells, respectively, were about 2-fold higher than those of PMA.

Fig. 3
Different effects of PDBu and PMA on HIV-1 replication in lymphocytic Molt-4 and monocytic THP-1 cells
Ordinate: zero value corresponds to the control (100%).

Discussion

We have investigated the effects of two phorbol ester derivatives, the hydrophilic PDBu and the hydrophobic PMA, on HIV-1 replication *in vitro*. Our results indicate that they probably act similarly; both stimulated HIV-1 replication in lymphocytic but not monocytic cells. Thus, the results suggest that these phorbol esters acted via similar mechanisms of regulation of HIV replication, namely through the PKC activation. Moreover, we propose that af-
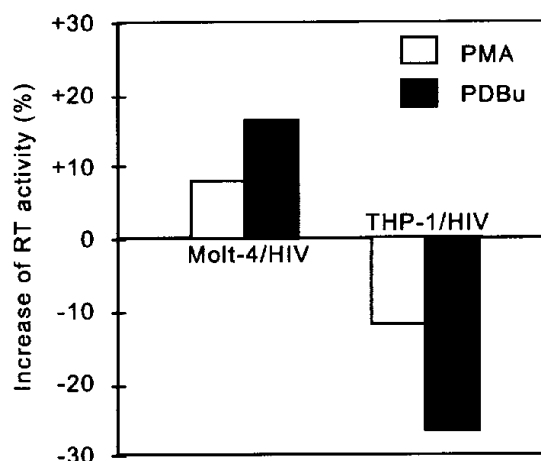
ter the initial induction of HIV-1 replication by the phorbol esters through PKC an additional "auto-enhancement" may also occur.

HIV-1 requires an active NF-kB complex for efficient transcription (Nabel and Baltimore, 1987). It is possible that after the stimulation of NF-kB by phorbol esters and reaching certain level of virus-specific proteases, the latter begin to process directly the cellular precursor of NF-kB (Rivere *et al.,* 1991), and thus increase the amount of active nuclear NF-kB complex. Thus both external and internal stimulatory factors could synergistically stimulate the LTR-directed HIV-1 gene expression.

In contrast to the results obtained with human lymphocytic cells used in this study, neither PMA nor PDBu stimulated the replicationof HIV-1 in monocytic cells. It is known from previous studies with monocytic U-937 cells (Firestein *et al.,* 1988) that more than 99% of untreated monocytic cells expressed CD4 receptors, however, the percentage of CD4^{+} cells decreased below 20% after incubation with 10^{-8} mol/l PMA. As CD4 receptors are intimately involved in HIV-1 infection, an alteration in the expression of this antigen could potentially affect the growth of infective virus; we can suggest that this may be one of the reasons for the low HIV-1 replication in our experiments. Another reason for the different growth of HIV-1 in lymphocytic and monocytic cells after the phorbol esters treatment may be a different PCK isozyme function in these cells. The biological significance of PKC heterogeneity is not known, but we suppose that individual PKC isozymes phosphorylate different substrates and consequently mediate different biological responses.

In spite of the similarity in the effects of PDBu and PMA, quantitative differences between the two derivatives were observed. PDBu behaved as a stronger stimulator or inhibitor of HIV-1 replication as compared to PMA. The efficacy of PDBu in Molt-4 and THP-1 cells was about two-fold higher. However, the precise mechanisms of the PKC activation by PMA and PDBu are not yet clear. Nevertheless, we assume that the hydrophilicity and the lower molecular mass of PDBu may be the reasons for the observed enhancing effect. It is also possible that PDBu acts directly in the cytoplasm as a calcium mobilizing agent. The raise in cytosolic Ca^{2+} is generally associated with biodegradation of phosphatidylinositol diphosphates to diacyl glycerol and inositol triphosphate and is thought to be involved in the transduction of a wide variety of cellular signals including the activation of PKC system.

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