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Gö 6976, a selective inhibitor of protein kinase C, is a potent antagonist of human immunodeficiency virus 1 induction from latent/low-level-producing reservoir cells *in vitro*

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ABSTRACT **Human immunodeficiency virus (HIV-1) infection is followed by a period of latency or a low-level-persistent (LLP) state that results in an asymptomatic infection of the host. Productive viral expression may be triggered by a variety of activators including mitogens, antigens, and cytokines. Protein kinase C (PKC) has been shown to be important in the intracellular cascade of signals induced by such activators. With U1 and ACH-2 cell lines representative of an HIV-1 postintegration state, the effect of Gö 6976, a synthetic inhibitor of PKC was tested. Gö 6976 is a nonglycosidic indolocarbazole found to potently inhibit HIV-1 induction by Bryostatin 1, tumor necrosis factor α, and interleukin 6. Gö 6976 effectively blocks viral transcription induced by Bryostatin 1 or tumor necrosis factor α that leads to the inhibition of intracellular viral protein synthesis and viral shedding. Gö 6976 also blocks interleukin 6-mediated posttranscriptional induction of viral proteins. The IC_{50} of Gö 6976 shows a 12- to 60-fold more potent effect than for H-7, another PKC inhibitor with a similar mechanism. The inhibitory effect is reduced when Gö 6976 is not added before or within 1 hr of induction by the potent PKC activator Bryostatin 1. However, U1 cells can be grown for long periods in a nontoxic concentration of Gö 6976 (300 nM), which confers virtual inhibition of HIV-1 induction without the development of resistance. Results indicate that inhibition of HIV-1 proviral induction from latent/low-level-producing infectious states with potent PKC inhibitors like Gö 6976 may represent an additional and promising antiviral approach.**

Acute human immunodeficiency virus (HIV-1) infection leads to a period of rapid viral replication followed by viremia that results in infection of 1% or more of circulating T lymphocytes, the primary target of the virus (1). Viremia is transient, however, because the cells productively infected with HIV-1 are removed from the circulation by an effective host immune response that results in a 10- to 100-fold decrease in the HIV-1-infected T cells (1). Unfortunately, no effective therapy yet exists for preventing infection after exposure. Thus, although the initial host response is effective in reducing and controlling HIV-infected cell numbers, it is not sufficient to prevent the postintegration latent or low-level-persistent (LLP) asymptomatic infections from occurring in host reservoir cells, such as circulating $CD4^+$ T lymphocytes (2) and monocyte/macrophages (3). Thus, the ultimate pathogenic effects of HIV-1 are not prevented and, after induction from the latent or LLP state, AIDS develops.

Induction of viral expression from the latent proviral or LLP state can be triggered by various cellular factors including mitogens (4–7), antigens (1), and cytokines (6–8), all of which activate the host cell. Therefore, latency and cellular activation are tightly linked (32). *In vitro* correlates of HIV-1 latency or LLP have been created in both monocyte and T-cell lines infected with HIV-1 (4, 5). The HIV-1 provirus contained in these cells can be induced by various cellular activators (4–8). Thus, these chronically infected cells provide useful models for dissecting the mechanism(s) of action of potential physiologic inducers or drugs that may affect this process.

We have previously discovered evidence linking protein kinase C (PKC) activation to HIV-1 infection in T-cell clones *in vitro* (10). Using two postintegration HIV-1-infected cell lines, U1, a U937 promonocytic-derived latently infected line (4), and ACH-2, a T-cell line (5), we have evaluated the effect of Gö 6976, a potent synthetic nonglycosidic indolocarbazole inhibitor of PKC on induction from latency/LLP *in vitro*.

MATERIALS AND METHODS

Reagents and Cytokines. Gö 6976 was synthesized as described (11). Human recombinant tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6) were obtained from Boehringer Mannheim. HA1004 and H-7 were purchased from Seikagaku America (St. Petersburg, FL). Gö 6976 was synthesized as described (11). Bryostatin 1 was from G. R. Pettit (Arizona State University), and the HIV-1 antiserum was from P. Pitha (The Johns Hopkins Oncology Center; ref. 12). The antiserum to HIV-1 p25/24 gag was obtained from the AIDS Research and Reference Reagent Program (Rockville, MD). All other materials were obtained from commercial sources.

Cell Culture. The HIV-1 latently infected T-cell line, ACH-2 (5), and the U1 promonocytic cell line (4) were obtained from the AIDS Research and Reference Reagent Program. Cytotoxicity was determined by using trypan blue vital dye exclusion.

Reverse Transcriptase (RT) Assay. Culture supernatants were assayed for the presence of HIV RT activity as described (13) with the following modifications: 20 μl of culture supernatant was added to 50 μl of RT mixture that contained deoxythymidine tri[^{32}P]phosphate (dTT^{32}P, Amersham) and incubated for 2 hr at 37°C. Twenty microliters of the mixture was spotted onto DE81 ion-exchange chromatography paper (Whatman), air-dried, and washed three times in 2× standard saline citrate. Cerenkov counting was performed on dried filters.

Immunoblotting of Viral Proteins. Cells at 3×10^5 cells per ml were treated with Gö 6976 for 1 hr before addition of an activator. The cells were washed in ice-cold phosphate-

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Abbreviations: LLP, low-level-persistent; PKC, protein kinase C; HIV-1, human immunodeficiency virus 1; RT, reverse transcriptase; TNF-α, tumor necrosis factor α; IL-6, interleukin 6; CC_{50}, concentration of drug producing 50% cytotoxicity; TR, therapeutic ratio.

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buffered saline (PBS) and lysed in PBS containing 1% Triton X-100 (Sigma), leupeptin at 20 μg/ml, aprotinin at 1.9 μg/ml, 1 mM phenylmethylsulfonyl fluoride for 60 min at 4°C. The lysates were centrifuged at 12,000 rpm for 10 min at 4°C, and the protein content of the resulting extracts was determined by using the BCA kit (Pierce). Samples (100 μg of protein) were resolved by SDS/PAGE on 12% gels, transferred to nitrocellulose membranes, and blotted with a 1:1000 dilution of HIV-1 immune serum obtained from an AIDS patient. The HIV-1 proteins were detected by using ^{125}I-labeled staphylococcal protein A (30 mCi/mg; Amersham; 1 Ci = 37 GBq).

Northern Blot Analysis. Total cellular RNA was prepared by the acid guanidinium thiocyanate–phenol/chloroform extraction method (9). Purified RNA (10 μg) was electrophoretically separated on a 6% formaldehyde/1% agarose gel and transferred to a nylon membrane (Bio-Rad). HIV-1-specific transcripts were detected by using the pJM105 insert, labeled via the random priming method, which contains the HIV-1 long terminal repeat and a portion of the gag region (14).

***In Vitro* Protein Kinase Assays.** One unit of protein kinase activity represents 1 pmol of phosphate incorporated per min. PKC (C-Kinase) was purified from rat brain (15). The conditions used for PKC and the other kinase activities were as described (16). For cAMP-dependent protein kinase (A-Kinase), the assay was done with the commercially available catalytic subunit (Sigma) in 50 μl containing 50 mM Pipes·NaOH (pH 7.5), 10 mM $MgCl_2$, 1 mM dithiothreitol, 50 μg of histone VII-S (Sigma), 40 μM ATP (0.3 μCi of [γ-^{32}P]ATP), 20 units of A-Kinase, and test compound. cGMP-dependent protein kinase (G-Kinase) was purified from bovine lung (17). The incubation mixture in 50 μl contained 20 mM Tris·HCl (pH 7.4), 5 mM $MgCl_2$, 40 μg of bovine serum albumin, 2% glycerol, 10 μM cGMP, 10 μg of histone II-A (Sigma), 10 μM ATP (0.3 μCi of [γ-^{32}P]ATP), 10 units of enzyme, and test compound. Both myosin light-chain and myosin light-chain kinase (M-Kinase) were purified from chicken gizzard (18, 19). The incubation mixture was 50 mM Mops·NaOH, pH 7.2/5 mM $MgCl_2$/100 μM ATP (0.3 μCi of [γ-^{32}P]ATP)/enzyme at 200 pmol/ml/test compound. Small resting B cells from murine spleen cells were used to isolate a tyrosine-specific protein kinase (T-Kinase) (20). The mixture in 50 μl contained 50 mM Tris·HCl (pH 7.4), 100 mM $MgCl_2$, 20 μM vanadate, 10 mM $MnCl_2$, 0.1% Nonidet P-40, 0.3 mM poly(Glu-Tyr), 4:1 (Sigma), 60 μM ATP (0.3 μCi of [γ-^{32}P]ATP), 10 units of enzyme, and test compound.

RESULTS

Effect of Gö 6976, Gö 6352, H-7, and HA1004 on HIV Induction by Bryostatin 1. Bryostatin 1 is an activator of cellular PKC (21). Recently we have synthesized a nonglycosidic indolocarbazole derivative, Gö 6976, that is a potent inhibitor of PKC *in vitro* (i.e., IC_{50} is 20 nM; Table 1 and Fig. 1) (11). This compound is structurally related to staurosporine, which is the most potent PKC inhibitor described to date (22). The effect of Gö 6976 on HIV-1 induction from latency in U1 or ACH-2 cells was examined. Induction was detected by measuring viral RT released from cells incubated with Bryostatin 1 (Fig. 2 and Table 2). Cells were incubated for 1 hr with increased concentrations of Gö 6976 or an inactive congener, Gö 6352, or the PKA inhibitor HA1004 followed by addition of the inducers. After 48 hr the viral RT released into the growth medium was quantitated. Results indicate that there is a dose-dependent inhibition of induction of viral RT by Gö 6976, whereas neither Gö 6352 nor HA1004 had a significant effect. The effect of Gö 6976 is potent, having an IC_{50} of 50–100 nM for U1 cells and 125–250 nM for ACH-2 cells (Fig. 2 and Table 2). The inhibitory effect measured was not due to a direct inhibition of HIV RT by the drug because >10 μM Gö 6976 could be added to supernatant from Bryostatin 1-induced U1 or ACH-2 cells with no effect on the RT activity measured (data not shown). By contrast, the IC_{50} for the PKC inhibitor H-7 (23) was 3–4 μM (Fig. 2). This result represents about a 30- to 60-fold less potent effect for H-7 in U1 cells and a 16- to 24-fold less potent effect in ACH-2 cells than for Gö 6976. Simultaneously, the cytotoxic effect of either Gö 6976 or H-7 on cell viability was measured. After incubation in the presence of both inducer and inhibitor, the concentration of drug producing 50% cytotoxicity (CC_{50}) was determined by vital dye exclusion to be >5 μM for Gö 6976 and 15 μM for H-7 in both U1 and ACH-2 cells. A therapeutic ratio (TR) of CC_{50}/IC_{50} can be calculated from the cytotoxicity and efficacy results. A TR for Gö 6976 of 50–100 in U1 and 20–40 in ACH-2 cells versus 4–5 for H-7 in either U1 or ACH-2 cells indicates that Gö 6976 has a significantly less steep toxicity range *in vitro* (Table 1).

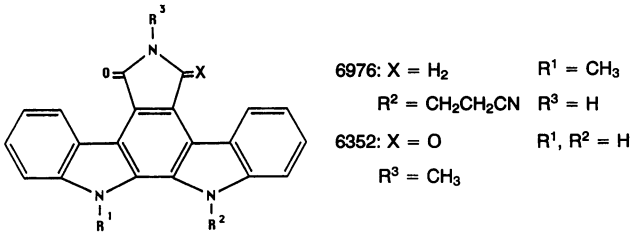

FIG. 1. Structures of Gö 6976 and Gö 6352.

Table 1. Inhibition of protein kinase

Compound	Kinase, IC_{50} in μM				
	C	A	G	M	T
Gö 6976	0.020	>100	6.2	5.8	>10
Gö 6352	>1	>10	>10	>30	>10
H-7	20	36	7.0	420	NT

C, PKC; A, cAMP-dependent protein kinase; G, cGMP-dependent protein kinase; M, myosin light-chain kinase; T, tyrosine-specific protein kinase from murine B cells; NT, not tested.

Gö 6976 Inhibits HIV-1 Induction by Physiologic Cellular Activators. Because Gö 6976 is an effective inhibitor of Bryostatin 1-induced viral RT, it was important to test the effect on potential physiologic activators (4–7). By using TNF-α and IL-6, Gö 6976 was found to inhibit RT induction in a dose-dependent manner. For TNF-α the IC_{50} is the same as that for Bryostatin 1, whereas the IC_{50} for IL-6 is at least 2-fold lower (Fig. 2; Table 2).

Gö 6976 Inhibits Induction of Viral Proteins and HIV-1 Transcription. Immunoblotting of total intracellular proteins indicates that Gö 6976 can inhibit viral protein synthesis in a dose-dependent manner after induction by Bryostatin 1, TNF-α, or IL-6 (Fig. 3). The IC_{50} for inhibition of viral proteins, determined by densitometry (data not shown), is essentially the same as that for viral RT (Table 2).

Northern analysis was done on total cellular RNA extracted from cells incubated with various concentrations of Gö 6976 and then induced with Bryostatin 1 or TNF-α. The findings indicate that Gö 6976 can inhibit transcription of both nonspliced and multiply spliced transcripts in a dose-dependent manner. Complete and >95% inhibition of steady-state transcripts, as judged by densitometry, was obtained with 250 nM Gö 6976 after induction by Bryostatin 1 or TNF-α, respectively (Fig. 4). However, as reported for IL-6 (8), no increase in steady-state levels of viral transcripts could be detected after 96 hr (data not shown). Thus, the inhibitory effects of Gö 6976 may occur at both the transcriptional and posttranscriptional levels depending upon the cytokine inducer used.

Effect of Gö 6976 Added Before or After Addition of Bryostatin 1. To determine at what time relative to addition

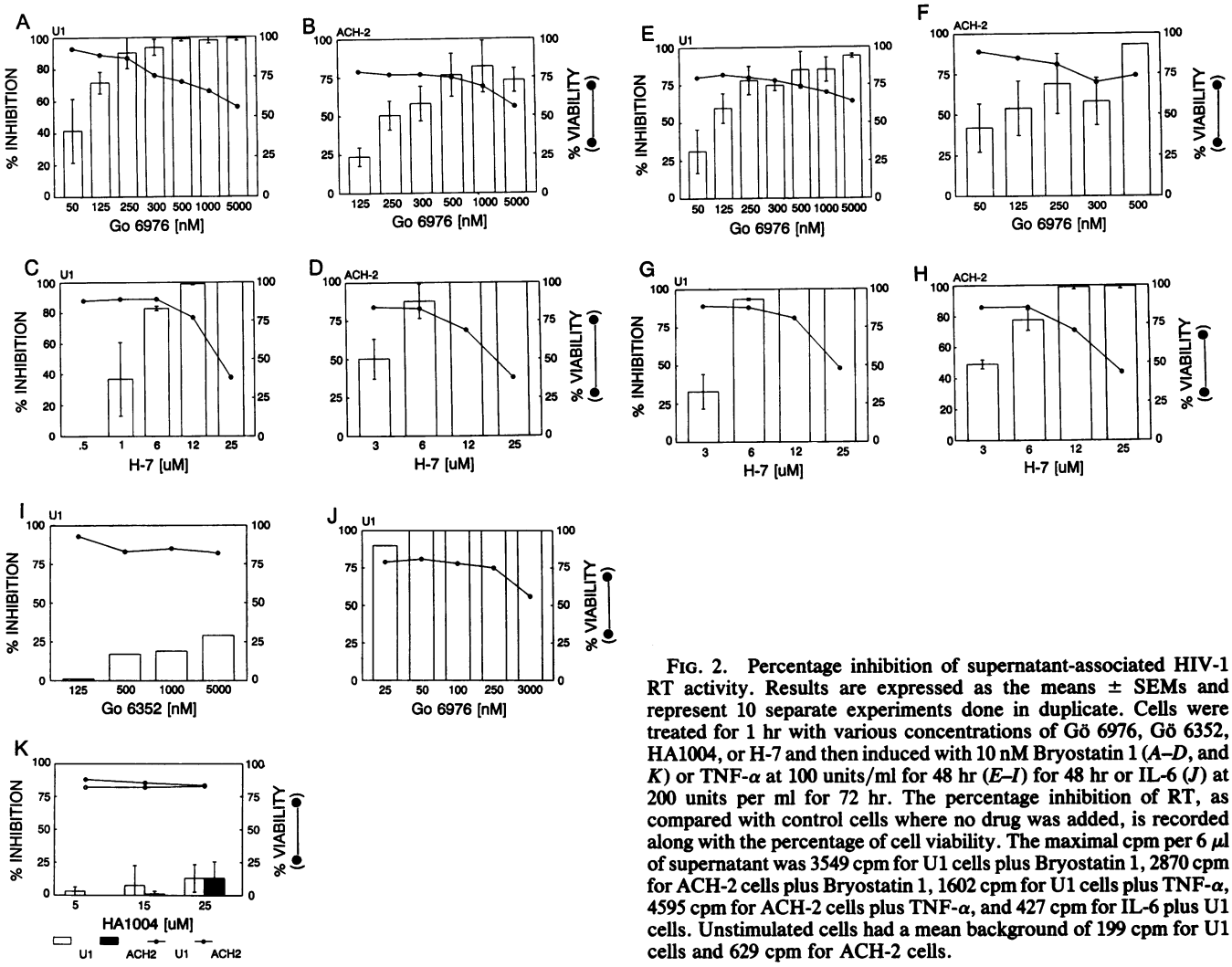

FIG. 2. Percentage inhibition of supernatant-associated HIV-1 RT activity. Results are expressed as the means ± SEMs and represent 10 separate experiments done in duplicate. Cells were treated for 1 hr with various concentrations of Gö 6976, Gö 6352, HA1004, or H-7 and then induced with 10 nM Bryostatin 1 (*A–D*, and *K*) or TNF-α at 100 units/ml for 48 hr (*E–I*) for 48 hr or IL-6 (*J*) at 200 units per ml for 72 hr. The percentage inhibition of RT, as compared with control cells where no drug was added, is recorded along with the percentage of cell viability. The maximal cpm per 6 μl of supernatant was 3549 cpm for U1 cells plus Bryostatin 1, 2870 cpm for ACH-2 cells plus Bryostatin 1, 1602 cpm for U1 cells plus TNF-α, 4595 cpm for ACH-2 cells plus TNF-α, and 427 cpm for IL-6 plus U1 cells. Unstimulated cells had a mean background of 199 cpm for U1 cells and 629 cpm for ACH-2 cells.

of the inducer Gö 6976 must be added to efficiently inhibit viral induction, the drug was added for 1 hr before or up to 8 hr after addition of Bryostatin 1. Immunoblotting results indicate that total viral protein induction is efficiently inhibited when Gö 6976 is added before the activator (Fig. 5). Although the inhibitory effect is also significant when the drug is added as late as 1 hr, even later additions fail to block induction. These results indicate that the effect of PKC activation on HIV-1 induction is rapid and essentially irreversible after 1–3 hr.

Effect of Gö 6976 on HIV Induction in Cells Chronically Exposed to Gö 6976. The above results suggest that continuous exposure of cells to Gö 6976 might be required to ensure inhibition of HIV-1 induction. However, whether cells could be grown for long periods in the continuous presence of Gö 6976 and whether resistance to the drug manifested by a loss of potency for inhibition of HIV-1 induction would develop required testing. U1 cells were grown for >3 mo in 300 nM Gö 6976, a concentration that can inhibit viral induction but that demonstrates little cytotoxicity in short-term assays (Fig. 2). The cells were washed and split, and fresh drug was added every 72 hr. After Gö 6976 incubation for 96 days, the cells were washed, and fresh drug was added 60 min before the activator. After 48 hr the cells were harvested, and total cellular protein was analyzed for viral p24 protein. In the absence of readdition of Gö 6976 to the washed cells, the induction of HIV-1 p24 protein was essentially identical whether or not the cells had been grown in Gö 6976 (Fig. 6). Any residual inhibitory effect of Gö 6976 that may have resulted from chronic exposure and/or intracellular accumulation was apparently effectively removed by washing. However, the Gö 6976 addition resulted in as potent an inhibitory effect as seen in cells not chronically exposed to Gö 6976. Thus, the IC_{50}, determined by plotting the densitometry

Table 2. Effect of protein kinase inhibitors on HIV-1 induction

Compound	Inducer	U1 cells			ACH-2 cells		
		IC_{50}	CC_{50}	TR	IC_{50}	CC_{50}	TR
Gö 6976	Bryostatin 1	50–100 nM	>5000 nM	50–100	125–250 nM	>5000 nM	20–40
	TNF-α	50–100 nM	>5000 nM	50–100	125–250 nM	>5000 nM	20–40
	IL-6	<25 nM	>5000 nM	200			
H-7	Bryostatin 1	3–4 μM	15 μM	4–5	3 μM	15 μM	4–5
	TNF-α				3–4 μM	15 μM	4–5
Gö 6352	TNF-α	>5 μM	>5 μM		>5 μM	>5 μM	
HA1004	Bryostatin 1	>50 μM			>50 μM		

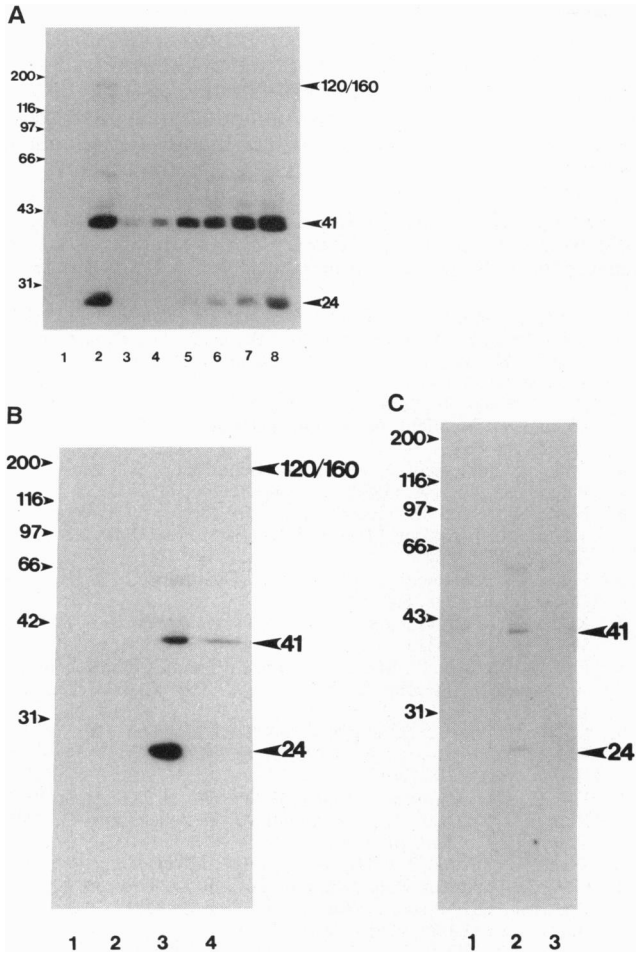

FIG. 3. Gö 6976 inhibits HIV-1-induced viral protein expression in U1 cells. Cells were treated for 1 hr with various concentrations of Gö 6976 and were then stimulated with 10 nM Bryostatin 1 (48 hr) (*A*), TNF-α (48 hr) at 100 units/ml (*B*), or IL-6 (96 hr) at 200 units/ml (*C*). The cells were harvested and washed; 100 μg of total cellular protein was analyzed for HIV-specific proteins, as described. (*A*) Lanes: 1, untreated cells; 2, Bryostatin 1-stimulated cells; 3, 500 nM Gö 6976 plus Bryostatin 1; 4, 250 nM Gö 6976 plus Bryostatin 1; 5, 125 nM Gö 6976 plus Bryostatin 1; 6, 50 nM Gö 6976 plus Bryostatin 1; 7, 25 nM Gö 6976 plus Bryostatin 1; 8, 10 nM Gö 6976 plus Bryostatin 1. (*B*) Lanes: 1, parental U937 cells plus Bryostatin 1; 2, untreated U1 cells; 3, TNF-α-stimulated cells; 4, 300 nM Gö 6976 plus TNF-α. (*C*) Lanes: 1, untreated U1 cells; 2, IL-6-stimulated cells; 3, 250 nM Gö 6976 plus IL-6. Numbers at left represent M_r markers ($\times 10^{-3}$); numbers at right represent viral protein weight ($\times 10^{-3}$).

results and then extrapolating, was estimated at 50–100 nM (Fig. 6). These findings indicate that U1 cells can be grown for prolonged periods in the presence of Gö 6976 without developing drug resistance.

DISCUSSION

Our results indicate that Gö 6976, a member of another class of PKC inhibitors, is a potent antagonist of induction of HIV-1 from the latent and/or LLP proviral state *in vitro*. The inhibitory effect of Gö 6976 appears specific for PKC because neither an inactive congener, Gö 6352, nor HA1004, a relatively specific inhibitor of a distinct protein kinase, protein kinase A, has an effect on viral induction. Gö 6976 is structurally related to staurosporine, the most potent inhibitor of PKC described (22). Gö 6976, staurosporine, and H-7 are competitive inhibitors of ATP binding to the catalytic domain of PKC (11, 21–23). These PKC inhibitors appear to block the rapid and direct activation of PKC in the cells by Bryostatin 1 and also any apparent indirect activation by

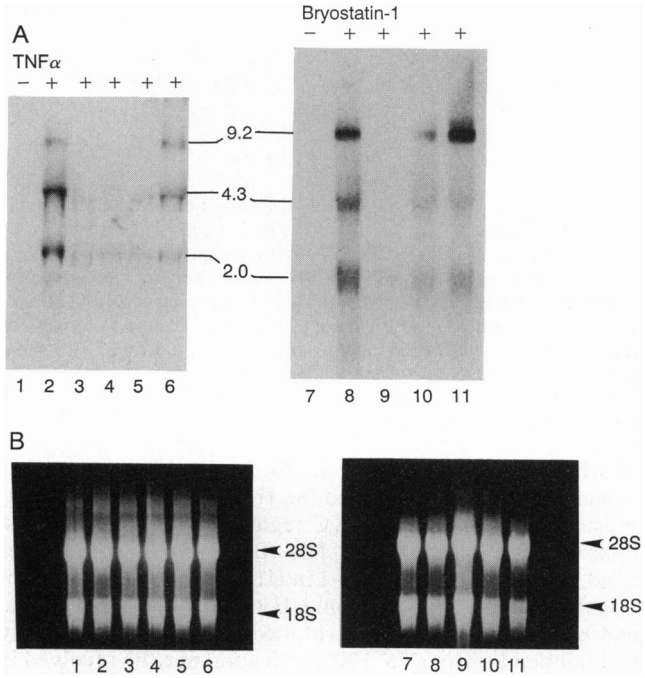

FIG. 4. Gö 6976 inhibits induction of HIV-1-specific transcripts. U1 cells were incubated with various concentrations of Gö 6976 for 1 hr before addition of either Bryostatin 1 (10 nM) or TNF-α (100 units per ml). Ten micrograms of total cellular RNA was analyzed as described. (*A*) Lanes: 1, untreated; 2, TNF-α stimulated; 3, 300 nM Gö 6976 plus TNF-α; 4, 250 nM Gö 6976 plus TNF-α; 5, 100 nM Gö 6976 plus TNF-α; 6, 50 nM Gö 6976 plus TNF-α; 7, untreated; 8, Bryostatin 1-stimulated; 9, 250 nM Gö 6976 plus Bryostatin 1; 10, 100 nM Gö 6976 plus Bryostatin 1; 11, 50 nM Gö 6976 plus Bryostatin 1. (*B*) Total RNA loaded per lane was visualized by ethidium bromide staining.

TNF-α (7, 24, 25) or IL-6 (8). After addition of Gö 6976 to cells, the effect of the pharmacologic or physiologic HIV-1 inducers on viral transcription is blocked, inhibiting viral replication. Functional PKC has been shown to be required for activation of a reporter gene containing the Tat promoter (26), and recently H-7 has also been reported to inhibit HIV-1 transcription in U1 cells (7). Furthermore, induction by

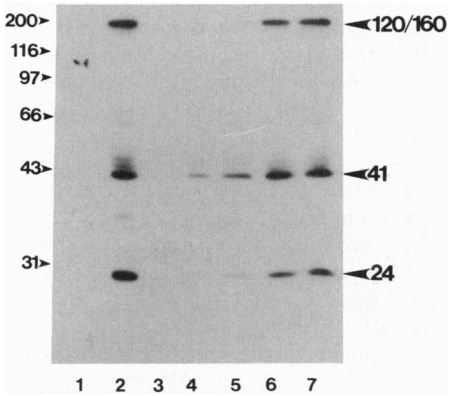

FIG. 5. Effect of Gö 6976 addition on induction of HIV-1-specific proteins. U1 cells were treated for various times before or after addition of 10 nM Bryostatin 1. One hundred micrograms of total cellular protein was analyzed by immunoblotting. Lanes: 1, untreated; 2, 10 nM Bryostatin 1 stimulated; 3, 1-hr incubation with 250 nM Gö 6976 before Bryostatin 1 addition; 4, Bryostatin 1 and 250 nM Gö 6976 added together; 5, Bryostatin 1 added 1 hr before Gö 6976 (250 nM); 6, Bryostatin 1 added 4 hr before Gö 6976 (250 nM); 7, Bryostatin 1 added 8 hr before Gö 6976 (250 nM). M_r markers ($\times 10^{-3}$) and viral proteins ($\times 10^{-3}$) are indicated.

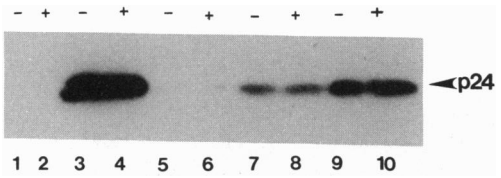

FIG. 6. Effect of Bryostatin 1 on HIV induction from latency in U1 cells after long-term exposure to Gö 6976. U1 cells were grown in 300 nM Gö 6976 for 96 days (+) or had no prior exposure to Gö 6976 (−). The cells were washed, and Gö 6976 was either added or not added for 1 hr before adding 10 nM Bryostatin 1. Cells were harvested 48 hr after Bryostatin 1 addition, and 100 μg of total cellular protein was analyzed for p24 HIV-1 core antigen, as described. Lanes: 1 and 2, unstimulated; 3 and 4, Bryostatin 1 stimulated; 5 and 6, 500 nM Gö 6976 plus Bryostatin 1; 7 and 8, 250 nM Gö 6976 plus Bryostatin 1; 9 and 10, 125 nM Gö 6976 plus Bryostatin 1.

phorbol esters (27) and TNF-α (28, 29) has been reported to enhance binding of NF-κB to the HIV long terminal repeat, presumably the result of PKC regulating association of the inhibitory IκB protein with NF-κB (30, 31). On the other hand, IL-6 can activate HIV-1 in U1 cells by a posttranscriptional mechanism (8). Because both HIV-1 transcriptional and posttranscriptional control mechanisms can apparently be inhibited by Gö 6976, PKC activation may be involved in both processes. Interestingly, the IC_{50} of Gö 6976 for IL-6 as compared with TNF-α or Bryostatin 1 is ≈2-fold lower. These differences could then reflect the presumed different stimulatory roles for PKC on induction. Therefore, it is not surprising that inhibition of PKC in cells latently infected with HIV-1 can profoundly affect induction by various cytokines and mitogens. Alternatively, although Gö 6976 appears highly specific for PKC *in vitro*, its *in vivo* spectrum may include other protein kinase-signaling cascades as well.

Our findings indicate that Gö 6976 appears relatively nontoxic. A TR (CC_{50}/IC_{50}), which takes into account both the efficacy for inhibition of viral induction and cytotoxicity of the drug, indicates that Gö 6976 is ≈30–60 and 12–24 times more potent than H-7 for inhibiting HIV-1 induction in U1 or ACH-2 cells, respectively (compare IC_{50} values in Table 2). Furthermore, Gö 6976 has a less steep toxicity slope (i.e., higher TR), indicating a more promising therapeutic potential.

Our results suggest that PKC activation occurs rapidly during the viral induction process. Thus, once cells are exposed to a potent PKC activator such as Bryostatin 1 for only a brief period (i.e., 60 min), little or no subsequent inhibitory effect on viral replication can be accomplished with Gö 6976. Because Bryostatin 1 can rapidly activate PKC after its addition to cells (21), the effectiveness of Gö 6976 is reduced when the drug is not present before or within 60 min of Bryostatin 1 addition. Therefore, to effectively block viral induction by such potent activators, the cells may need continual exposure to Gö 6976. Importantly, we found that U1 cells can be grown in Gö 6976 at a nontoxic concentration (i.e., 300 nM) that can effectively block HIV-1 induction. Furthermore, after prolonged growth in the presence of Gö 6976, the cells remain equally sensitive to the inhibitory effect of Gö 6976 (i.e., IC_{50} = 50–100 nM), as compared with cells grown in the absence of drug (Fig. 6). Therefore, the potential for development of cellular resistance, which could represent a problem for any therapeutic application, may be quite low for this drug.

Together these findings suggest that inhibition of PKC activation may represent another approach to preventing HIV-1 induction from the postintegration latent or LLP state that exists in host reservoir cells. To this end, Gö 6976, a member of another class of PKC inhibitors, may represent a potent and promising addition to our therapeutic armamentarium.

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