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Adenallene and cytallene: Acyclic nucleoside analogues that inhibit replication and cytopathic effect of human immunodeficiency virus *in vitro*

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ABSTRACT **Although several antiretroviral compounds are already known, almost no acyclic nucleoside derivatives lacking an oxacyclopentane have been reported to exert significant inhibition against human immunodeficiency virus type 1 (HIV-1) *in vitro*. We found two unsaturated acyclic nucleoside derivatives, adenallene [9-(4′-hydroxy-1′,2′-butadienyl)adenine] and cytallene [1-(4′-hydroxy-1′,2′-butadienyl)cytosine], that protect various CD4$^+$ T-cell lines from the infectivity and cytopathic effect of HIV-1. These compounds inhibit the expression of HIV-1 *gag*-encoded protein and suppress viral DNA synthesis at concentrations that do not affect functions of normal T cells *in vitro*. They also inhibit the *in vitro* infectivity of another human retrovirus, HIV-2. Further *in vitro* analyses of the anti-HIV-1 activity revealed that the presence of two cumulated double bonds between the 1′ and 2′ carbons and between the 2′ and 3′ carbons confers antiretroviral activity in certain pyrimidine or purine derivatives containing a four-carbon chain. We have also found that the 4′-hydroxyl group is critical for the *in vitro* anti-HIV activity of adenallene. Our observations may provide structure–activity relationships for acyclic nucleoside analogues and may be of value in developing a new class of experimental drugs for the therapy of HIV-related diseases.**

In the relatively short time since the first clinical recognition of acquired immunodeficiency syndrome (AIDS), a great deal has been learned about the life cycle of human immunodeficiency virus (HIV) as well as the treatment of the disease (1–4). However, much remains to be learned in devising strategies for treating this disease.

We tested a number of nucleoside analogues that lack an oxacyclopentane moiety for *in vitro* anti-HIV-1 activity and found that adenallene and cytallene§ could inhibit the infectivity, replication, and cytopathic effect of HIV-1 and HIV-2 *in vitro*. In this paper we also describe a possible structure–antiretroviral activity relationship in such acyclic nucleoside analogues. These observations could have theoretical and clinical implications in the strategy to develop new anti-HIV drugs.

MATERIALS AND METHODS

Viruses and Cells. HIV-1 and HIV-2 were pelleted by ultracentrifugation from the culture supernatants of HTLV-III$_B$-producing H9 cells (2) and HIV-2-producing CEM cells (5) and prepared to contain 5.9×10^{10} and 2.6×10^{11} virus particles per ml, respectively. In the assay for inhibition of HIV cytopathic effect (see below), 0.5 and 0.005 virus particle per cell represented the minimum cytopathic doses of the virus preparations of HIV-1 and HIV-2, respectively. A CD4$^+$ T-cell clone (ATH8) and a normal, CD4$^+$, tetanus toxoid-specific T-cell clone (TM11) as well as H9 cells were used as target cells for infection by HIV. Characteristics of clones ATH8 and TM11 have been described (6–8). Cell cultures were not synchronized as to cell cycle.

Reagents. Adenallene [9-(4′-hydroxy-1′,2′-butadienyl)adenine] and cytallene [1-(4′-hydroxy-1′,2′-butadienyl)cytosine] were synthesized as described (9). Detailed procedures will be published elsewhere. All tested compounds, except guanallene [9-(4′-hydroxy-1′,2′-butadienyl)guanine], were >95% pure as shown by nuclear magnetic resonance spectra. Guanallene contained ≈10% [9-(4′-hydroxy-2′-butynyl)guanine] (**18** in Table 1), which is inactive against HIV-1. Compounds **5**, **6**, **7**, **8**, **13**, **17**, and **19** in Table 1 have been described (9–11), whereas compounds **3**, **4**, **11**, **12**, **15**, **16**, **18**, **21**, and **23** were newly synthesized and will be reported elsewhere (S.P. and J.Z.). Adenallene, cytallene, guanallene, and hypoxallene [9-(4′-hydroxy-1′,2′-butadienyl)hypoxanthine] are racemic mixtures [50% *R* and 50% *S* (enantiomers); Fig. 1]. 3-Azido-3′-deoxythymidine (N_3ddThd, popularly known as AZT) was kindly provided by the Wellcome Research Laboratories. 2′,3′-Dideoxyadenosine (ddAdo) and 2′,3′-dideoxycytidine (ddCyd) were provided by the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute.

Assay of Inhibition of HIV Cytopathic Effect. Inhibition was assayed as described (6). Clone TM11 cells were stimulated by 0.6 limiting flocculation unit of tetanus toxoid (Commonwealth of Massachusetts Department of Public Health, Jamaica Plain, MA) per ml and irradiated autologous peripheral blood mononuclear cells (PBMCs) and were cultured in complete medium [RPMI 1640 supplemented with 4 mM L-glutamine, 15% (vol/vol) undialyzed and heat-inactivated fetal bovine serum, and 50 units of penicillin and 50 μg of streptomycin per ml] containing 15% (vol/vol) interleukin 2 (IL-2, lectin-depleted; Advanced Biotechnologies, Silver Spring, MD) and 25 units of recombinant IL-2 (Amgen Biological, Thousand Oaks, CA) per ml for 6 days before assay. ATH8 cells were used without the antigen stimulation. Target T cells (2×10^5) were exposed to HIV for 1 hr,

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Abbreviations: N_3ddThd, 3′-azido-3′-deoxythymidine (3′-azido-2′,3′-dideoxyribosylthymine, commonly known as AZT); ddAdo, 2′,3′-dideoxyadenosine; ddCyd, 2′,3′-dideoxycytidine; HIV, human immunodeficiency virus; HIV-1 and HIV-2, HIV types 1 and 2; IL-2, interleukin 2; PBMC, peripheral blood mononuclear cell; PHA, phytohemagglutinin; PWM, pokeweed mitogen.

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§Suggested trivial names are based on nomenclature of nucleic bases and a suffix, allene: the adenine derivative with an allene group is designated adenallene, the cytosine derivative cytallene (see Fig. 2), the guanine derivative guanallene, and the hypoxanthine derivative hypoxallene.

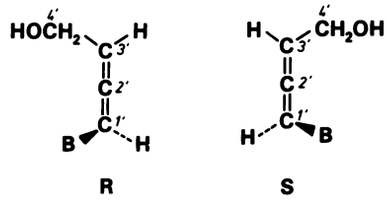

FIG. 1. Structures of allenic derivatives with nucleic acid bases. B denotes a base such as adenine, cytosine, guanine, or hypoxanthine. All the allenic analogues used were racemic mixtures of *R* and *S* enantiomers.

resuspended in 2 ml of fresh complete medium with IL-2, and incubated at 37°C in humidified air containing 5% CO_2. Control cells were treated similarly but were not exposed to the virus. At various time points, viable cells were counted in a hemocytometer by the trypan blue-exclusion method.

Determination of HIV-1 *gag*-Encoded Protein. HIV-1 *gag* (group-specific antigen) expression was assessed as described (6). In brief, H9 cells (2×10^4) were exposed to HIV-1 (2000 viral particles per cell), resuspended, and cultured at 37°C in humidified air containing 5% CO_2. On days 6, 7, and 9, the percentage of the H9 cells expressing p24 *gag* protein was determined by indirect immunofluorescence with anti-HIV-1 p24 murine monoclonal antibody M26 (12).

Southern Blot Hybridization. Techniques were as described (13). High molecular weight DNA was extracted with organic solvents, and 40 μg of such DNA was digested with endonuclease *Asp*718 (Boehringer Mannheim). The digests were subjected to electrophoresis, the separated DNA fragments were transferred to nitrocellulose and hybridized with a radiolabeled insert of a molecular clone of the *env* region of HIV-1 (strain BH10) containing a 1.3-kilobase *Bgl* II fragment, and the viral DNA was detected by autoradiography. Relative levels of the detected viral DNA were compared by densitometry (X-Rite 301; X-Rite, Grand Rapids, MI) of the exposed film (7).

Assay of Antigen- or Mitogen-Induced T-Cell Activation. Washed responder TM11 cells (10^5) were cultured for 3 days with tetanus toxoid and 10^5 irradiated autologous PBMCs in 200 μl of complete medium. In some experiments, 10^5 fresh PBMCs were cultured with or without phytohemagglutinin (PHA) or pokeweed mitogen (PWM) for 3 days. All cultured cells were exposed to 0.5 μCi of [*methyl*-^{3}H]thymidine (25.8 Ci/mmol; 1 Ci = 37 GBq) for the final 12 hr and then harvested onto glass fibers for quantitation of the incorporated radioactivity by scintillation counting.

Table 1. *In vitro* anti-HIV-1 activity of acyclic nucleoside analogues

Compound*	Concentration, μM	Protective effect,† %	Cytotoxicity,‡ %
Adenine derivatives			
(1) 2',3'-Dideoxyadenosine	10, 100, 1000	100, 90, 68	0, 9, 29
(2) $HOCH_2CH{=}C{=}CH$-adenine (*R,S*) (adenallene)	10, 100, 1000	54, 97, 14	6, 3, 70
(3) $ClCH_2CH{=}C{=}CH$-adenine (*R,S*)	1, 10, 100	0, 0, 0	5, 18, 100
(4) $HOCH_2CH{=}CHCH_2$-adenine (*Z*)	10, 100, 200, 500	0, 0, 3, 0	2, 17, 52, 76
(5) $HOCH_2C{\equiv}CCH_2$-adenine	1, 10, 100	8, 0, 15	9, 0, 18
(6) $ClCH_2C{\equiv}CCH_2$-adenine	1, 10, 100	0, 0, 0	3, 45, 69
(7) $HOCH_2CH_2CH_2CH_2$-adenine	1, 10, 100, 1000	10, 9, 16, 0	21, 0, 17, 72
(8) $HOCH_2CH_2CH(OH)CH_2$-adenine (*R,S*)	1, 10, 100	0, 6, 0	0, 13, 38
Cytosine derivatives			
(9) 2',3'-Dideoxycytidine	0.2, 0.5, 1, 5	58, 100, 92, 83	0, 0, 3, 8
(10) $HOCH_2CH{=}C{=}CH$-cytosine (*R,S*) (cytallene)	0.2, 0.5, 1, 5	68, 76, 90, 66	6, 0, 4, 32
(11) $HOCH_2CH{=}CHCH_2$-cytosine (*E*)	0.1, 1, 10, 100	12, 0, 0, 0	0, 0, 27, 14
(12) $ClCH_2CH{=}CHCH_2$-cytosine (*E*)	1, 10, 100	12, 0, 0	17, 6, 100§
(13) $HOCH_2C{\equiv}CCH_2$-cytosine	0.1, 1, 10, 100	0, 0, 0, 8	0, 0, 0, 44
Guanine derivatives			
(14) 2',3'-Dideoxyguanosine	5, 10, 100, 500	100, 100, 99, 48	2, 0, 5, 50
(15) $HOCH_2CH{=}C{=}CH$-guanine (*R,S*) (guanallene)	1, 10, 100, 500	0, 2, 0, 0	3, 0, 45, 100§
(16) $HOCH_2CH{=}CHCH_2$-guanine (*Z*)	10, 100, 500, 1000	1, 1, 4, 4	4, 0, 51, 51
(17) $HOCH_2CH{=}CHCH_2$-guanine (*E*)	1, 10, 100, 200	6, 0, 5, 8	0, 0, 3, 0
(18) $HOCH_2C{\equiv}CCH_2$-guanine	0.1, 1, 10, 100	2, 0, 3, 0	0, 0, 0, 0
(19) $HOCH_2CH_2CH(OH)CH_2$-guanine (*R,S*)	1, 10, 100	10, 16, 0	12, 12, 0
Thymine derivatives			
(20) 2',3'-Dideoxythymidine	10, 50, 100	4, 14, 68	16, 9, 13
(21) $HOCH_2CH_2C(=O)CH_2$-thymine	1, 10, 100	0, 22, 0	0, 0, 8
Hypoxanthine derivatives			
(22) 2',3'-Dideoxyinosine	1, 10, 100, 1000	7, 97, 100, 46	9, 6, 0, 40
(23) $HOCH_2CH{=}C{=}CH$-hypoxanthine (*R,S*) (hypoxallene)	1, 10, 100, 1000	2, 0, 2, 2	0, 0, 0, 0

ATH8 cells (2×10^5) were exposed to HIV-1 ($HTLV\text{-}III_B$, 2000 viral particles per cell) and cultured in the presence of the compounds designated above. Total viable cells were counted on day 6 or 7. Orders of numbers in the column for concentrations correspond to the orders of numbers in other columns. The anti-HIV-1 activities of 2',3'-dideoxynucleosides are shown for reference.

*(*R,S*), a racemic mixture [50% *R* and 50% *S* form (enantiomers)]; (*Z*), conformation in which the base is *cis* relative to the 4' carbon; (*E*), conformation in which the base is *trans* relative to the 4' carbon.

†Percent protective effect of a compound on the survival and growth of ATH8 cells exposed to the virus was determined by the following formula: 100 × [(number of viable cells exposed to HIV-1 and cultured in the presence of the compound) − (number of viable cells exposed to HIV-1 and cultured in the absence of the compound)]/[(number of viable cells cultured alone) − (number of viable cells exposed to HIV-1 and cultured in the absence of the compound)]. By this formula, when the number of viable cells exposed to the virus and compound is the same as or more than the number of viable cells cultured alone, 100% is given. Calculated percentages ≤0 are expressed as 0%.

‡Percent cytotoxicity of a compound on the growth of ATH8 cells was determined by the following formula: 100 × [1 − (number of total viable cells cultured in the presence of the compound)/(number of total viable cells cultured alone)]. Calculated percentages ≤0 are expressed as 0%.

§Dimethyl sulfoxide was used as diluent for these compounds. At high concentrations the observed toxicities apparently resulted from toxicities of both the drug and the diluent.

RESULTS

Adenallene and Cytallene Inhibit Replication of HIV-1 *in Vitro*. We tested more than 30 nucleoside derivatives that lacked an oxacyclopentane moiety for *in vitro* anti-HIV-1 activity as assayed by inhibition of cytopathic effect. Representative data are summarized in Table 1.

We found two acyclic nucleoside derivatives, adenallene (**2** in Table 1) and cytallene (**10** in Table 1), that exerted a potent anti-HIV-1 activity *in vitro*. In the absence of drugs, by day 7 after exposure to HIV-1, almost all ATH8 cells were killed by the virus (Fig. 2). However, 10 μM adenallene exerted a partial protective effect on ATH8 cells, and at 100 μM, the number of viable cells in cultures exposed to HIV-1 was equal to that of unexposed control cultures. At higher concentrations, the drug appeared to be somewhat more toxic to cell growth than the reference compound ddAdo (Fig. 2 and Table 1). Cytallene at ≥0.5 μM gave virtually complete protection to ATH8 cells exposed to the virus (Fig. 2). Under the conditions used, cytallene appeared to be as effective as N_3ddThd against HIV-1 *in vitro*. However, the capacity of both adenallene and cytallene to nullify the cytopathic effect of HIV-1 was often lost by day 14 of culture, whereas the reference compounds ddAdo, ddCyd, and N_3ddThd remained effective against the virus through the 14-day period

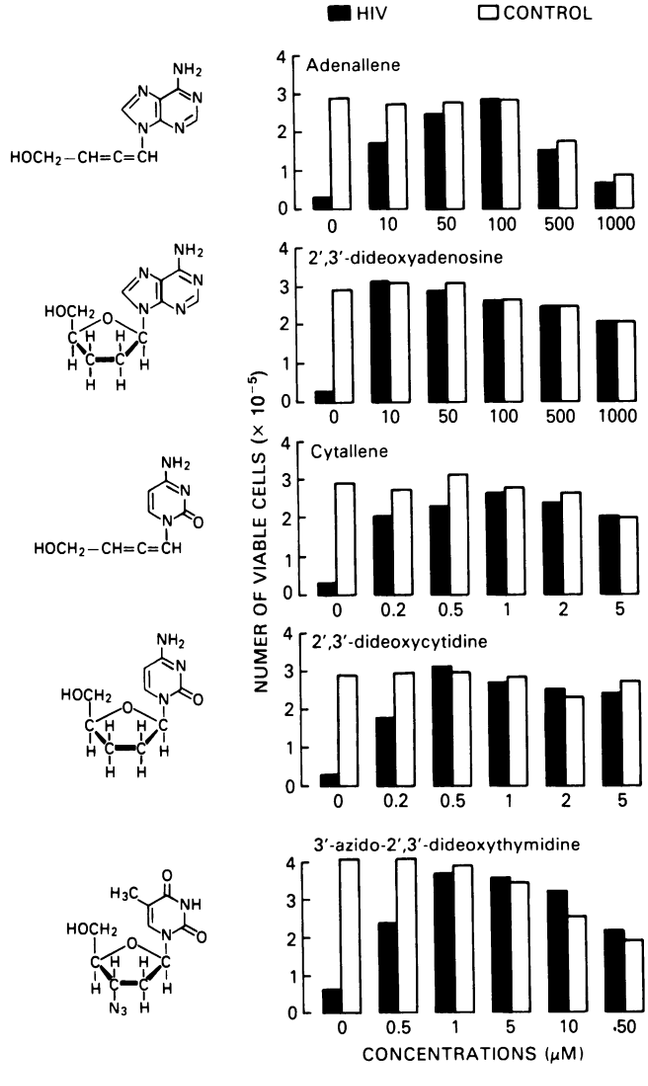

FIG. 2. Inhibition of the infectivity and cytopathic effect of HIV-1 in ATH8 cells by adenallene and cytallene. Total viable cells were counted on day 7.

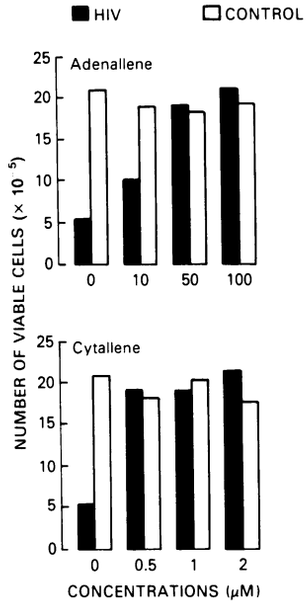

FIG. 3. Inhibition of the infectivity and cytopathic effect of HIV-1 by adenallene and cytallene in a normal helper/inducer T-cell clone (TM11). TM11 cells (2×10^5) were first exposed to soluble tetanus-toxoid and accessory cells in the presence of IL-2, 6 days before assay. They were then exposed to HIV-1 ($HTLV\text{-}III_B$; 5000 viral particles per cell) and cultured in the presence of adenallene or cytallene. Total viable cells were counted on day 15.

(data not shown). Unlike 2',3'-dideoxyinosine [which is a metabolite of ddAdo and possesses potent activity against HIV-1 *in vitro* as well (8)], the corresponding acyclic compound hypoxallene was neither effective against the virus nor toxic to the cells even at concentrations up to 1000 μM (Table 1). Interestingly, the corresponding guanine-containing acyclic derivative, guanallene, was not active against HIV-1 in the ATH8 system. This relative inactivity of hypoxallene or guanallene to work against the virus was confirmed both in the cytopathic-effect inhibition assay and in the HIV-1 *gag* expression assay at various multiplicities of infection (2000, 1000, 200, and 100 virus particles per cell).

The protective effects of adenallene and cytallene were confirmed in a different target-cell system, the normal helper/inducer T-cell clone TM11. In the absence of the drug, HIV-1 exerted a substantial cytopathic effect on the TM11 population by day 15 in culture, resulting in an ≈75% decrease in the number of viable cells (Fig. 3). However, addition of 50 or 100 μM adenallene or addition of 0.5–2 μM cytallene completely protected TM11 cells without affecting cell growth.

Adenallene and Cytallene Inhibit HIV-1 *gag* Expression. By day 9 after exposure of $CD4^+$ H9 cells to HIV-1 (2000 virus particles per cell), ≈45% of the cells expressed *gag*-encoded protein p24 (Fig. 4). In this system, adenallene at 50 μM gave partial inhibition, and at 100 μM it completely suppressed p24 expression by day 7; however, viral breakthrough was observed and the replication of the virus resumed in H9 cells by day 9. With 200 μM adenallene, however, essentially no H9 cells became positive throughout the 9 days of culture. Cytallene at 1 μM exhibited a partial protective effect, and at 5 μM, no positive cells were detected at any time during the culture period.

Inhibition of HIV-1 DNA Synthesis. We then asked whether viral DNA could be detected in susceptible ATH8 cells exposed to the virus but protected by acyclic nucleoside derivatives. In the absence of the drugs, viral DNA was readily detectable on day 1 and a substantial amount of the viral DNA was detected on day 3 (Fig. 5, lanes a and d). However, in the presence of 250 μM adenallene (lanes b and e) or 5 μM cytallene (lanes c and f), the amount of viral DNA was markedly reduced. Densitometry of the exposed film showed 88.7% and 88.7% decreases on day 1, and 96.6% and 91.3% decreases on day 3, in the amount of viral DNA from the ATH8 cells protected by adenallene and cytallene, respectively, as compared to viral DNA from unprotected cells.

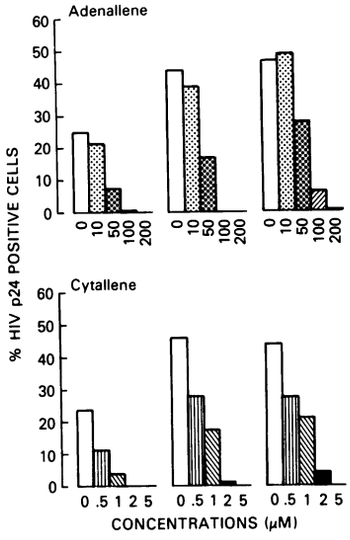

FIG. 4. Inhibition of expression of HIV-1 p24 *gag* protein in H9 cells by adenallene and cytallene. H9 cells were cultured in the presence or absence of various concentrations of adenallene or cytallene after exposure to HIV-1 ($HTLV\text{-}III_B$; 2000 viral particles per cell). On days 6 (*Left*), 7 (*Center*), and 9 (*Right*) in culture, the percentage of H9 cells expressing HIV-1 p24 *gag* protein was assessed by indirect immunofluorescence with anti-HIV-1 p24 murine monoclonal antibody.

Adenallene and Cytallene Protect Helper/Inducer T-Cells from HIV-2 Cytopathic Effects. We asked whether adenallene and cytallene could block the cytopathic effect of HIV-2. ATH8 cells were exposed to an exceedingly potent preparation of HIV-2 (20 virus particles per cell; 0.005 virus particle per cell represented the minimum cytopathic dose of the HIV-2 preparation). In the absence of the drug, the HIV-2 virions exerted a substantial cytopathic effect on ATH8 cells by day 6 (Fig. 6). Adenallene at ≥50 μM or cytallene at ≥5 μM completely suppressed the infectivity and cytopathic effect of HIV-2. When this experiment was repeated, we found that the protective effect of adenallene and cytallene against HIV-2 was roughly the same as that against HIV-1 under the conditions used.

Cumulated Double Bonds Are Required for *in Vitro* Anti-HIV-1 Activity of Acyclic Nucleoside Derivatives. We explored some of the structure–antiviral activity relationships for the acyclic nucleoside derivatives. We first asked whether the two cumulated double bonds were critical for anti-HIV-1 activity. We conducted experiments to determine whether

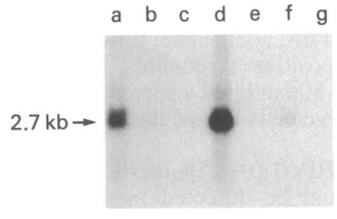

FIG. 5. Inhibition of HIV-1 DNA synthesis in ATH8 cells by adenallene or cytallene. ATH8 cells (10^7) were exposed to HIV-1 ($HTLV\text{-}III_B$; 1000 viral particles per cell) and cultured in the presence or absence of drugs. On days 1 (lanes a–c) and 3 (lanes d–f) after exposure to the virus, high molecular weight DNA was extracted. Lane g, DNA from ATH8 cells that were not exposed to the virus; lanes a and d, DNA from ATH8 cells that were exposed to the virus and cultured in the absence of drug; lanes b and e, DNA from ATH8 cells exposed to the virus but protected by 250 μM adenallene; lanes c and f, DNA from ATH8 cells protected by 5 μM cytallene. The viral DNA was detected as a 2.7-kilobase (kb) *env*-containing internal fragment.

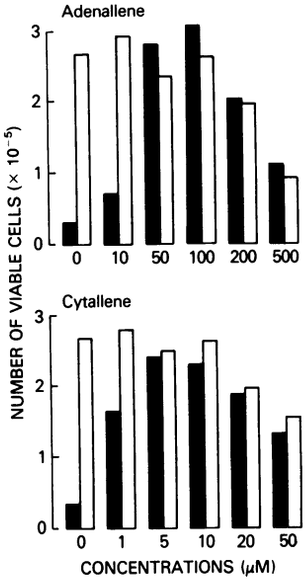

FIG. 6. Inhibition of the infectivity and cytopathic effect of HIV-2 in ATH8 cells by adenallene and cytallene. ATH8 cells (2×10^5) were exposed to an exceedingly potent HIV-2 preparation (20 viral particles per cell) and cultured in the presence of acyclic nucleoside analogues (solid bars). Control cells were similarly treated but were not exposed to the virus (open bars). Total viable cells were counted on day 6.

acyclic compounds that had only one double bond in the four-carbon chain (**4**, **11**, **12**, **16**, and **17** in Table 1) were effective against HIV-1 *in vitro*. These compounds tested failed to protect ATH8 cells against the cytopathic effect of HIV-1 at the concentrations tested. Compounds that had one triple bond in the four-carbon chain were not effective against the virus (**5**, **6**, **13**, and **18** in Table 1). All other acyclic compounds without the two-double-bond conformation failed to suppress the virus (Table 1).

We then asked whether the hydroxyl group at the 4′ carbon was necessary for anti-HIV-1 activity. To test this, we substituted a chlorine for the hydroxyl group of adenallene and obtained 4′-chloro-4′-deoxyadenallene (**3** in Table 1). This substitution nullified the capacity of adenallene to block the replication of HIV-1, indicating that the 4′-hydroxyl group is required for the antiviral activity.

Effect of Adenallene and Cytallene on Antigen- or Mitogen-Induced T-Cell Activation. Finally, we tested the effects of adenallene and cytallene on the *in vitro* immune reactions of TM11 cells and PBMCs from normal individuals (Fig. 7). Adenallene at concentrations up to 50–100 μM did not inhibit antigen-induced proliferation or PWM- or PHA-induced T-cell activation. Proliferation of antigen-activated normal TM11 cells and PWM-activated PBMCs was unaffected by cytallene at concentrations up to 10 μM. Proliferation of PHA-activated PBMCs was moderately suppressed by 10 μM cytallene.

DISCUSSION

In the current study, both adenallene and cytallene showed potent activity against HIV-1 and HIV-2 *in vitro*; however, some viral breakthrough was observed both in the ATH8-cell cytopathic-effect inhibition assay and in the H9-cell HIV-1 *gag* expression assay. Further, when we looked for the presence of HIV-1 proviral DNA, certain amounts of viral genome were detected in ATH8 cells protected by these compounds. We (14) and others (15) have observed a similar resumption of HIV-1 replication or escape of the virus in certain cell systems in the presence of N_3ddThd. The antiviral effects of such drugs depend not only on an adequate concentration of the nucleosides but also on adequate levels of certain kinases that are cell-cycle-dependent. The apparent resumption of viral replication in the presence of adenallene and cytallene might perhaps be due to catabolism of these compounds or to infection of certain cell populations (which then in turn produce more virus for variable periods)

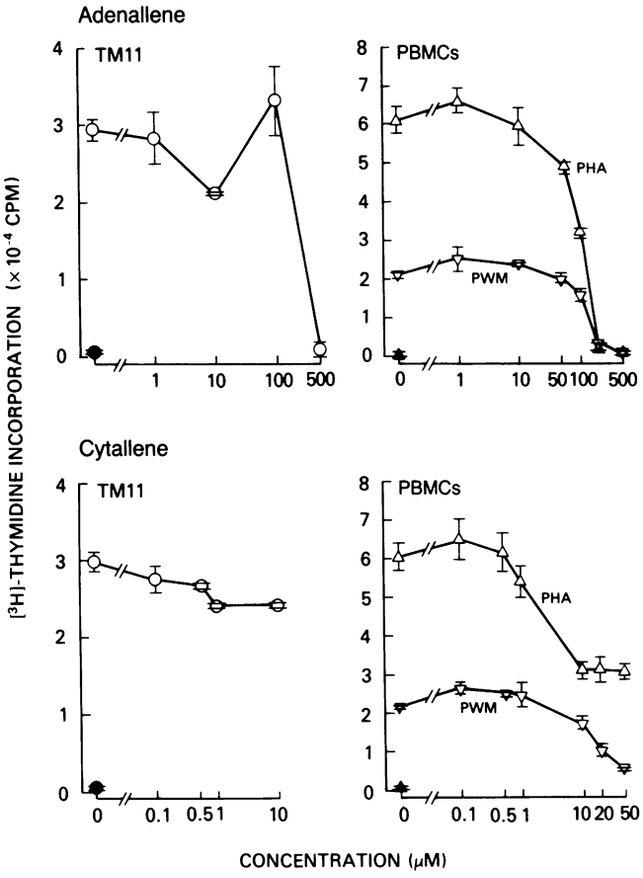

FIG. 7. Effect of adenallene and cytallene on antigen- or mitogen-induced proliferation of normal T cells. Various concentrations of adenallene or cytallene were added to TM11 cells that were stimulated with soluble tetanus-toxoid (*Left*) or to PBMCs from a healthy individual that were stimulated with PHA (△) or PWM (▽) (*Right*). Proliferation of T cells was assessed as incorporation of [^{3}H]thymidine. Each solid symbol denotes the background [^{3}H]thymidine incorporation when the responder cells were cultured alone in the absence of adenallene, cytallene, antigen, or mitogen. Data are expressed as the means ± 1 SD of triplicated determinations.

that have an insufficient capacity to activate the drugs at a given point in time. The precise way in which HIV-1 resumes replication in certain culture systems in the presence of these drugs is not established and will require further research.

Unlike 2′,3′-dideoxyinosine, which can give rise to 2′,3′-dideoxyadenosine 5′-triphosphate inside human cells and which exerts a potent anti-HIV effect *in vitro* (8), hypoxallene was neither effective against the virus nor toxic to the cells even at 1 mM. Guanallene also was not active against HIV-1 in our study. These data suggest that hypoxallene and guanallene might not be good substrates for relevant cellular kinases or that they are poor inhibitors for target viral enzyme(s) (e.g., reverse transcriptase) in spite of intracellular activation.

We have found that both adenallene and cytallene are resistant to pH 1 at room temperature for at least 16 hr (unpublished data). The stability of these drugs at low pH might make them potentially suitable for regimens that involve prolonged therapy, since experience with other nucleosides, including N_3ddThd (16) and ddCyd (17), suggests that adenallene and cytallene will be absorbed by oral administration. Taken together, our observations may be of value in developing a new class of experimental drugs for the therapy of HIV-related diseases.

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