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Anti-HIV activity of adefovir (PMEA) and PMPA in combination with antiretroviral compounds: in vitro analyses

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Abstract

Adefovir (PMEA, 9-(2-phosphonomethoxyethyl)adenine), an acyclic nucleoside phosphonate analogue is active against retroviruses, hepadnaviruses and herpesviruses. Adefovir dipivoxil, an orally bioavailable prodrug of adefovir is currently in phase III clinical trials for the treatment of HIV and phase II clinical trials for the treatment of HBV infections. PMPA (9-(2-phosphonomethoxypropyl)adenine) is a related acyclic nucleoside phosphonate analogue that has demonstrated potent anti-SIV activity in rhesus macaques and recently has shown marked anti-HIV activity in a phase I clinical study. Since the standard of care for AIDS patients has become combination therapy, the effects of other antiretroviral compounds (d4T, ddC, AZT, ddI, 3TC, nelfinavir, ritonavir, indinavir, and saquinavir) on the anti-HIV activity of adefovir and PMPA were investigated in vitro. Adefovir and PMPA both demonstrated strong synergistic anti-HIV activity in combination with AZT. Adefovir demonstrated minor to moderate synergistic inhibition of HIV replication in combination with PMPA, d4T, ddC, nelfinavir, ritonavir, and saquinavir. PMPA demonstrated minor synergistic inhibition of HIV replication in combination with ddI and nelfinavir (and adefovir). All other combinations showed additive inhibition of HIV replication in vitro. Importantly, no antagonistic interactions were measured for any of the adefovir or PMPA combinations. © 1997 Elsevier Science B.V.

Keywords: Adefovir dipivoxil; PMEA; PMPA; HIV; Drug combinations

1. Introduction

Monotherapy with reverse transcriptase inhibitors and protease inhibitors have proven effective in reducing viral load in vivo (Kuritzkes et al., 1995; Markowitz et al., 1995; Kitchen et al.,

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1995; Schapiro et al., 1996). However, due to the development of drug resistant variants, adverse event profiles, noncompliance, drug metabolism, or other less well defined reasons, monotherapy with these drugs typically becomes less effective over time (Hirsch and D'Aquila, 1993; de Jong et al., 1995). A recent approach taken to surmount the limitations of monotherapy has been dual or triple combination therapies that include reverse transcriptase (RT) inhibitors, protease inhibitors, or non-nucleoside RT inhibitors. The combination of antiviral agents that leads to a prolonged suppression of viral replication is likely to be due to additive or synergistic antiviral activity combined with a reduction in the opportunity for emergence of drug resistant viruses. These tenets have been clearly demonstrated for the combinations of 3TC and AZT (Kuritzkes et al., 1995; Larder et al., 1995), saquinavir and AZT (Jacobsen et al., 1996) and many other combinations (reviewed in de Jong et al., 1995). In addition, one component of a combination may select for a resistance suppressor mutation that resensitizes viruses to a second drug in the combination. The M184V, L74V, K65R, Y181C and W88G mutations each has been reported to independently restore wild type AZT susceptibility to some AZT resistant viruses carrying the AZT-associated resistance mutations (St. Clair et al., 1991; Larder, 1994; Richman et al., 1994; Mellors et al., 1996; Tachedjian et al., 1996).

Adefovir, 9-[2-(phosphonomethoxy) ethyl]adenine (PMEA), an acyclic nucleoside phosphonate analog, is active against retroviruses (Pauwels et al., 1988) and herpesviruses (De Clercq et al., 1986, 1987) in vitro and in vivo. Adefovir dipivoxil (bis-POM PMEA), an orally bioavailable prodrug of PMEA, has shown anti-HIV activity in early clinical trials (Deeks et al., 1996, 1997) and is currently in further clinical trials for the treatment of HIV and hepatitis B virus infections. Adefovir diphosphate (PMEApp), the active metabolite of adefovir, is a competitive inhibitor with regard to dATP and also serves as an alternate substrate for incorporation into viral DNA, functioning as a chain terminator of viral DNA synthesis (Cherrington et al., 1995).

PMPA, 9-[2-(phosphonomethoxy) propyl]adenine, like adefovir, is an acyclic nucleoside phosphonate analog. PMPA is active against HIV in vitro (Balzarini et al., 1991, 1993) and has demonstrated potent anti-SIV activity in models of acute and chronic infection of rhesus macaques (Tsai et al., 1995; Van Rompay et al., 1996). An oral prodrug of PMPA is currently under clinical evaluation for the treatment of HIV infection. PMPA functions to inhibit HIV replication by the same mechanisms as adefovir (Cherrington et al., 1995).

It is possible that during combination therapy, drug interactions may arise in vivo that would influence their antiviral activity. Therefore, to investigate these potential interactions in vitro, experiments involving combinations of adefovir or PMPA and other antiviral compounds were performed.

2. Materials and methods

A previously described 96 well XTT assay using MT2 cells infected with HIV IIIb (Gu et al., 1995; Cherrington et al., 1996) was used to determine the anti-HIV and cytotoxic activities of adefovir, PMPA, AZT, ddC, ddI, d4T, 3TC, nelfinavir, ritonavir, indinavir, and saquinavir. 2′,3′-Didehydro-3′-deoxythymidine (d4T) was obtained from Bristol Myers Squibb. 2′,3′-Dideoxy-3′-thiacytidine (3TC) was obtained from Glaxo Wellcome. Nelfinavir was obtained from Agouron Pharmaceuticals. Ritonavir was obtained from Abbott Laboratories. Indinavir was obtained from Merck Research Laboratories. Saquinavir was obtained from Roche. 3′-Azido-3′-deoxythymidine (AZT), 2′,3′-dideoxycytidine (ddC), and 2′,3′-dideoxyinosine (ddI) were purchased from Sigma (St. Louis, MO). Data from these experiments are shown in Table 1. All of the compounds exhibited potent anti-HIV activity and favorable selectivity indexes.

Adefovir and PMPA were each assayed for anti-HIV activity in combination with the five currently approved antiretroviral nucleoside analogs (AZT, ddC, ddI, d4T, and 3TC) and four protease inhibitors (nelfinavir, ritonavir, indi-

navir, and saquinavir). A 96 well plate format was used for the combination experiments. One drug was diluted by thirds vertically and the other drug by thirds horizontally with the IC_{50} concentration for each drug present in the middle of the dilution series. HIV (IIIb) infected MT2 cells were then added to the 96 well plate and on day 5 post-infection, an XTT assay was performed as previously described (Gu et al., 1995; Cherrington et al., 1996). All combinations were performed in triplicate. Cytotoxic effects of the drug combinations were determined as described for the antiviral assays except uninfected rather than infected MT2 cells were used. Concentrations of drugs used in the combination experiments were as follows: adefovir 0.6–60 μM, PMPA 1–100 μM, AZT 0.003–10 μM, other RT inhibitors 0.03–100 μM, and all protease inhibitors 0.0003–1 μM.

The antiviral combination data were analyzed by the MacSynergy II program (Prichard et al., 1993) as previously described (Mulato et al., 1996). Briefly, antiviral or cytotoxicity data generated from the XTT assay over a range of specific concentrations of two drugs were entered into the MacSynergy II program which calculated the synergistic/antagonistic interactions occurring at each drug concentration present in the combination (defined by 3-dimensional dose response surfaces and subtracting out additive interactions) and represented the data as a volume of synergy or antagonism. These volumes, in units of μM2%, are analogous to the units for area under a dose response curve in 2-dimensions (μM%). It has been shown previously that the MacSynergy II program (a 3-dimensional model) and other methods of 2-dimensional analyses of drug combination experiments are comparable (Prichard and Shipman, 1990; Mulato et al., 1996; Deminie et al., 1996).

Table 1
IC_{50} and CC_{50} values of antiviral compounds against HIV (IIIb)[a]

Compound	IC_{50} (μM)[b]	CC_{50} (μM)[c]	SI
Adefovir	13.4 ± 4.2	225 ± 25	17
PMPA	6.3 ± 3.3	$>500 \pm 0$	>79
AZT	0.17 ± 0.08	475 ± 35	2795
ddC	1.4 ± 0.90	400 ± 0	286
ddI	6.5 ± 1.5	$>500 \pm 0$	>121
d4T	8 ± 2.5	$>100 \pm 0$	>13
3TC	1.8 ± 0.25	$>500 \pm 0$	>278
Nelfinavir	0.006 ± 0.002	$>10 \pm 0$	>1667
Ritonavir	0.013 ± 0.001	$>10 \pm 0$	>769
Indinavir	0.004 ± 0.001	$>10 \pm 0$	>2500
Saquinavir	0.003 ± 0.001	$>10 \pm 0$	>3333

SI: selectivity index, defined as CC_{50} divided by IC_{50}.
[a] Assays were conducted as previously described (Gu et al., 1995; Cherrington et al., 1996). IC_{50} and CC_{50} values shown are means plus S.D. of at least two separate experiments.
[b] IC_{50} is defined as the concentration of drug that inhibits cell killing by virus infection by 50%.
[c] CC_{50} is defined as the concentration of drug that inhibits cell viability by 50%.

3. Results

The results of the adefovir combination experiments are shown in Fig. 1a. The combination of adefovir and AZT exhibited the strongest synergy with most of these interactions occurring at AZT concentrations of 0.3–3 μM and adefovir concentrations of 0.7–20 μM. The combination of adefovir and AZT has also demonstrated marked synergy (synergy volumes equivalent to or 2-fold higher than those measured in MT2 cells) in a second cell line, MOLT4 (data not shown). The combination of ddC with adefovir exhibited moderate synergy at ddC concentrations of 3–10 μM and adefovir concentrations of 0.3–10 μM. The combinations of PMPA, d4T, nelfinavir, ritonavir, or saquinavir with adefovir exhibited minor synergy, all occurring at concentrations that were within a half log of each drug's IC_{50} value. The combination of adefovir with ddI, 3TC or indinavir exhibited additive inhibition of HIV replication. No significant antiviral antagonism was measured for any of the adefovir combinations (Fig. 1a).

The antiviral data from four adefovir combinations were also analyzed using the fractional inhibitory concentration (FIC) method (Elion et al., 1954). FIC values < 1 suggests synergistic interactions. In all cases, the results using the FIC method were consistent with those reported in Fig. 1a using the MacSynergy II method. Specifi-

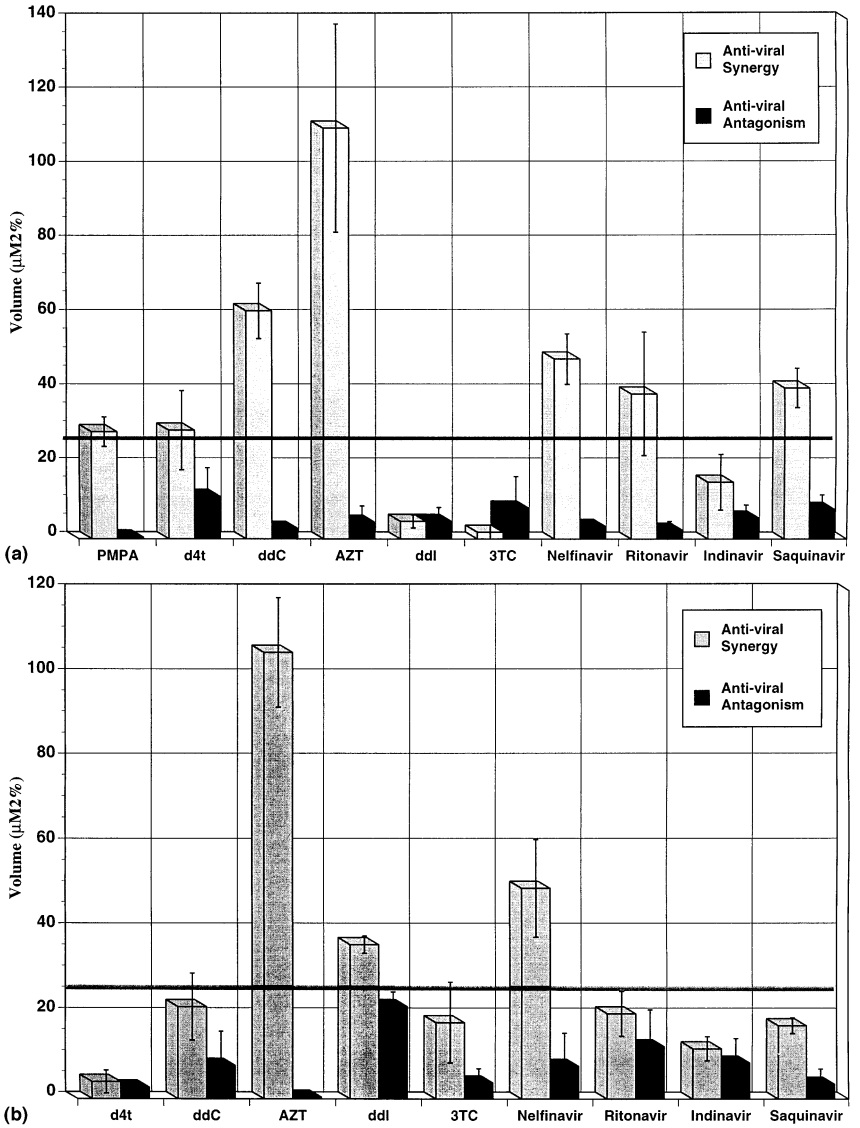

Fig. 1. Volumes of synergy (gray boxes) and antagonism (black boxes) were computed by the MacSynergy II program using a 95% confidence interval (Prichard et al., 1993) and are defined by the program as follows: values $< 25\ \mu M^2\%$ should be regarded as insignificant and are probably not important in vivo; values between 25 and 50 $\mu M^2\%$ should be considered minor; values between 50 and 100 $\mu M^2\%$ indicate moderate synergy/antagonism and may be important in vivo; values $> 100\ \mu M^2\%$ indicate strong synergy/antagonism and are likely to be important in vivo. Means and S.D. (vertical bars) from at least two independent experiments are shown. A horizontal bar is drawn at 25 $\mu M^2\%$: values $> 25\ \mu M^2\%$ indicate potentially significant synergistic interactions. (a) Antiviral activity of adefovir in combination with other anti-HIV compounds; (b) antiviral activity of PMPA in combination with other anti-HIV compounds.

cally, adefovir and d4T, adefovir and AZT, and adefovir and saquinavir showed synergistic inhibition of HIV replication (mean FIC values of 0.9, 0.8 and 0.8, respectively) while adefovir and indinavir showed additive inhibition of HIV replication (mean FIC value of 1.2).

PMPA in combination with the eight inhibitors above was assayed for anti-HIV activity in vitro. Results of these experiments are shown in Fig. 1b. Like adefovir, the combination of PMPA and AZT exhibited the strongest synergy. The majority of these synergistic interactions occurred at AZT concentrations of 0.1–3 μM and PMPA concentrations ranging from 0.3–30 μM. The combination of PMPA and nelfinavir exhibited moderate synergy primarily at nelfinavir concentrations of 0.001–0.1 μM and PMPA concentrations of 10–30 μM. PMPA in combination with ddI exhibited minor synergy primarily at ddI concentrations of 10–30 μM and PMPA concentrations of 0.3–1 μM. The combinations of PMPA with the remaining compounds exhibited additive inhibition of HIV replication. No significant antiviral antagonism was exhibited for any of the PMPA combinations (Fig. 1b). The antiviral data from four PMPA combinations were also analyzed using the fractional inhibitory concentration (FIC) method and the results using this method were consistent with those reported in Fig. 1b using the MacSynergy II method. Specifically, PMPA and AZT and PMPA and nelfinavir showed synergistic inhibition of HIV replication (FIC values of 0.3 and 0.5, respectively), while PMPA and d4T and PMPA and indinavir showed additive inhibition of HIV replication (FIC values of 1.3 and 1.2, respectively).

Additionally, the potential interactions that may occur among the different adefovir and PMPA combinations were analyzed in uninfected cells. No significant alterations in cell growth were measured for any of the combinations (data not shown).

4. Discussion

Combinations of reverse transcriptase inhibitors have been shown to decrease viral RNA load, increase CD4 cell number and in some cases, slow progression and prolong survival (reviewed in de Jong et al., 1995; Foudraine et al., 1997; Henry et al., 1997; Katlama et al., 1997). More recently, the triple drug combination involving two nucleoside analogs and a protease inhibitor have also shown significant clinical benefit (reviewed in de Jong et al., 1995; Cavert et al., 1997; Hirsch et al., 1997; Markowitz et al., 1997; Wong et al., 1997). The ultimate goal of combination therapy is to maintain suppression of viral replication. Therefore, it is important to consider potential drug interactions, metabolism of compounds in different cell types/body compartments, as well as resistance profiles of individual components of combination therapy.

Results of the in vitro drug combination experiments presented here suggest that adefovir and PMPA would function effectively as part of many anti-HIV combination therapeutic regimens. All of the drug combinations investigated here showed additive inhibition on viral replication and, in many cases, synergy. The data presented here also demonstrated the lack of any antagonistic interactions among the combinations with adefovir or PMPA.

Previous work has also shown that in contrast to some nucleoside analogs (AZT), adefovir and PMPA demonstrate potent anti-HIV activity in numerous cell lines including resting lymphocytes and monocyte/macrophages (Balzarini et al., 1991; Perno et al., 1996; Buckheit, personal communication). Recent data has shown that these cell types may be important reservoirs of viral replication, and indicate that combinations of compounds with complementing activity in different cell types should be considered (Haase et al., 1996; Perelson et al., 1996; Cavert et al., 1997). Furthermore, both adefovir and PMPA have favorable resistance profiles (Gu et al., 1995; Cherrington et al., 1996; Foli et al., 1996; Van Rompay et al., 1996; Cherrington et al., 1997; Mulato et al., 1997). Given the in vitro and in vivo anti-HIV properties of both of these molecules, further clinical investigation into the use of adefovir and PMPA in combination with other therapies is warranted.

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