0026-895X/95/010015-06$3.00/0

MOLECULAR PHARMACOLOGY, 48:15–20 (1995).

Inhibition of Human Immunodeficiency Virus Type 1 Replication by 7-Methyl-6,8-bis(methylthio)pyrrolo[1,2-*a*]pyrazine, an *In Vivo* Metabolite of Oltipraz

HANS J. PROCHASKA, WILLIAM G. BORNMANN, PENNY BARON, and BRUCE POLSKY

Molecular Pharmacology and Therapeutics Program (H.J.P., W.G.B.) and Infectious Disease Service (P.B., B.P.), Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Received December 12, 1994; Accepted April 20, 1995

SUMMARY

Oltipraz, an inhibitor of human immunodeficiency virus type 1 replication *in vitro* (ED_{50} ~ 10 μM), undergoes extensive metabolism *in vivo*. Most of the orally administered drug undergoes opening of the dithiolethione ring, reduction, recyclization, and methylation to form 7-methyl-6,8-bis(methylthio)pyrrolo[1,2-*a*]pyrazine ("metabolite III"). We report here that metabolite III inhibits viral replication *in vitro* (ED_{50} ~ 25 μM) in acutely infected H9 and CEM T cell lymphoma cell lines. Although both metabolite III and oltipraz were able to inhibit phorbol-12-myristate-13-acetate-stimulated viral replication in the chronically infected U1 promonocytic leukemia cell line, only metabolite III was able to inhibit phorbol-12-myristate-13-acetate-stimulated viral replication in chronically infected ACH-2 T cell lymphoma cells. The results with ACH-2 cells suggest that oltipraz inhibits an early stage of the viral life cycle, whereas metabolite III affects human immunodeficiency virus type 1 replication at a step distal to viral integration. This is consistent with the finding that oltipraz inhibits reverse transcriptase, whereas metabolite III does not. Although the mean ED_{50} for metabolite III in acutely infected peripheral blood mononuclear cells was 18 μM, the ED_{50} was below 5 μM in three of eight independent experiments. Studies of metabolite III in combination with oltipraz in acutely infected peripheral blood mononuclear cells demonstrated significant antiviral synergy. These results raise the possibility that the *in vitro* potency of oltipraz may underestimate its antiretroviral activity *in vivo*. Based on these results, the pharmacokinetics of oltipraz and metabolite III will be compared with the pharmacodynamic effects of orally administered oltipraz in a forthcoming phase I/II trial of oltipraz in patients with p24 antigenemia.

Oltipraz (5-pyrazinyl-4-methyl-1,2-dithiole-3-thione) (Fig. 1), which was developed and tested in humans as an antischistosomal agent, is a highly effective inhibitor of chemical carcinogenesis (1–3). The effectiveness of oltipraz in preventing neoplasia in a wide variety of experimental models has prompted a clinical evaluation of oltipraz as a potential human anticarcinogen (4–8). Our laboratories identified an additional biological activity of oltipraz; it is an inhibitor of HIV-1 replication (9). RT is a potential target, because oltipraz irreversibly inhibits RT in the template/primer binding domain (9). However, because oltipraz also inhibits viral replication in a chronic infection model (10), oltipraz appears to possess an additional antiviral mechanism. Interestingly, the potency of the drug in U1 cells is markedly potentiated by preincubation of the cells with oltipraz before PMA stimulation (10). These results led us to speculate that metabolites of oltipraz may also play a role in the inhibition of viral replication.

The metabolic fate of oltipraz is complex (7, 8, 11, 12). Less than 1% of the administered dose is excreted unaltered in the urine. The major metabolic pathway for oltipraz is likely to occur via a scenario involving opening of the 1,2-dithiole-3-thione ring by a nucleophile (perhaps glutathione), followed by a two-electron reduction of the adduct, elimination of the nucleophile, and recyclization (13). The resulting species is then methylated to form 7-methyl-6,8-bis(methylthio)pyrrolo[1,2-*a*]pyrazine (Fig. 1). 7-Methyl-6,8-bis(methylthio)pyrrolo[1,2-*a*]pyrazine, termed "metabolite III" by Bieder *et al.* (11), is the common precursor for 10 other, closely related metabolites (which differ primarily in the oxidation state of the sulfur atoms and the pyrazine ring). The amounts of rearranged metabolites isolated from human serum or urine were found to be much larger than the amount of oltipraz

This work was supported by the Al Tanenbaum Clinical AIDS Research Fund, institutional grants from Memorial Sloan-Kettering Cancer Center, and National Cancer Institute Cancer Center Support Grant NCI-P30-CA08748. H.J.P. is the recipient of an American Cancer Society Junior Faculty Research Award (JFRA-422).

ABBREVIATIONS: HIV-1, human immunodeficiency virus type 1; RT, reverse transcriptase; PBMC, peripheral blood mononuclear cell; PBS, phosphate-buffered saline; PHA-P, phytohemagglutinin P; IL-2, interleukin-2; DMSO, dimethylsulfoxide; MTT, 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide; PMA, phorbol-12-myristate-13-acetate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid.

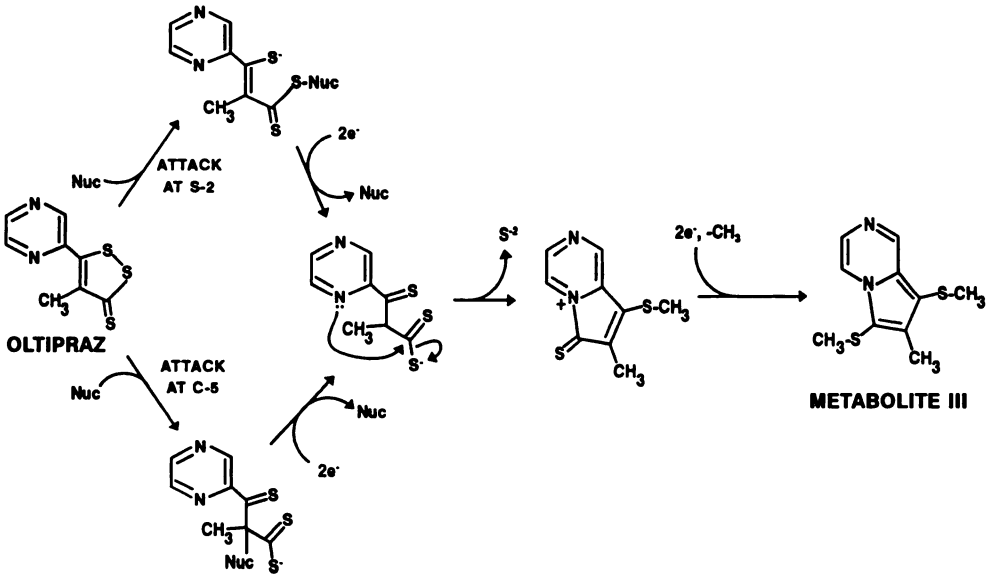

Fig. 1. Proposed routes for the biotransformation of oltipraz to metabolite III. The scheme was adapted from the work of Fleury *et al.* (13). *Nuc*, nucleophile.

(11). There are no known biological activities of metabolite III and related congeners.

Because there is evidence that oltipraz exerts its antiviral activity by more than one mechanism (10), we were interested in whether metabolites of this drug possessed antiviral activity. To address this question, we have tested oltipraz and metabolite III in H9, U1, ACH-2, and CEM cell lines, as well as in PBMCs. As noted above, the antiviral effects of oltipraz have been demonstrated in H9 (9) and PMA-stimulated U1 (10) cell lines, as models of acute and chronic HIV-1 replication, respectively. Because U1 cells are representative of a monocytoid lineage, we chose to examine ACH-2 cells as a model of chronic T cell infection. ACH-2 cells produce low levels of infectious virus under basal conditions but HIV-1 replication can be markedly induced with PMA (14). CEM cells were used not only because they represent another widely utilized model for acute viral replication in T cells but also because they are progenitors of A3.01 cells, from which the ACH-2 cell line was derived. Thus, it would be expected that differences in metabolism and uptake of oltipraz would be minimized between CEM and ACH-2 cells, rendering more valid a comparison of oltipraz and metabolite III in models of acute versus chronic viral replication. PBMCs were tested because they are the closest model of *in vivo* conditions. Establishing that metabolite III possesses antiviral activity at a step distal to viral integration and is synergistic with the parent compound (oltipraz) is the subject of this paper.

Experimental Procedures

Materials. Oltipraz was synthesized as described (15). PBMCs were obtained from the New York Blood Center as buffy coats; cell lines were obtained from the National Institutes of Health AIDS Research and Reference Reagent Program (Rockville, MD). PHA-P was obtained from Difco Laboratories (Detroit, MI), purified human IL-2 was purchased from Schipparelli Biosystems (Columbia, MD), interferon-α was from Hoffman-LaRoche (Nutley, NJ), Ficoll was obtained from Organon Technica (Durham, NC), and 0.2-mm silica gel/aluminum-backed thin layer chromatography plates were purchased from E. Merck (Darmstadt, Germany). All other materials were obtained from the sources cited (9, 10) or were purchased from Sigma Chemical Co. (St. Louis, MO) or Fisher (Springfield, NJ). NMR spectra were recorded with a Bruker AMX400 spectrometer, IR spectra were recorded with a Perkin Elmer 1600 series Fourier transform-IR spectrometer, and mass spectra were recorded with a Delsi Nermag Automass high performance gas chromatograph-mass spectrometer.

Synthesis of metabolite III. Metabolite III was prepared according to the method of Fleury *et al.* (13). Briefly, oltipraz (0.11 g, 0.50 mmol) was dissolved in 500 ml of absolute ethanol under argon and heated to 35°, yielding a deep orange-colored solution. While this mixture was being stirred, 20 ml of a yellow-brown solution of absolute ethanol containing 5 mmol of sodium ethoxide and 0.30 g (2.5 mmol) of L-cysteine were added via hypodermic syringe over 5 min. Although there was no obvious change in the color of the reaction, thin layer chromatography revealed complete disappearance of oltipraz 1 hr after addition of the sodium ethoxide/cysteine. After an additional 1 hr of stirring, 3.1 ml (7.1 g, 50 mmol) of CH_3I were added via hypodermic syringe. There was an immediate change in color from deep orange to lemon yellow. After the reaction was allowed to proceed for an additional 1 hr, the flask was removed from the oil bath, and small portions of crushed dry ice were added to neutralize the reaction mixture. The ethanol was removed by rotary evaporation, and the resulting yellow paste was partitioned between water and ethyl acetate. After the organic fraction was dried over $MgSO_4$, the ethyl acetate was removed by rotary evaporation. The resulting orange oil was dissolved in toluene, applied to a silica column (2.5 × 10 cm), and eluted with toluene/acetone (98:2). The pooled fractions were dried by rotary evaporation and yielded 0.10 g (91% yield) of straw-colored solid [m.p., 62–63° (uncorrected); literature m.p., 66°; ^{1}H NMR (400 MHz, $CDCl_3$), δ 2.213 (s, 3 H, C_8-SCH_3), δ 2.274 (s, 3 H, C_6-SCH_3), δ 2.489 (s, 3 H, C_7-CH_3), δ 7.6852 (d, J = 4.8 Hz, 1 H, C_3-H), δ 8.188 (dd, J = 4.8 and 1.4 Hz, 1 H, C_4-H), and δ 8.962 (d, J = 1.4 Hz, 1 H, C_1-H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 10.45 (C_7-CH_3), δ 17.64 (C_8-SCH_3), δ 20.31 (C_6-SCH_3), δ 107.68 (C_8), δ 115.64 (C_7), δ 116.07 (C_4), δ 128.27 (C_3), δ 131.02 (C_{8a}), δ 135.76 (C_6), and δ 142.86 (C_1); IR (KBr pellet), 2917.9, 1604.2, 1498.5, 1435.4, 1419.7, 1370.6, 1336.7, 1370.6, 1336.7, 1306.7, 1288.7, 1230.8, 1117.5, 968.9, 949.3, 709.7, 721.9, 662.4, 595.8, and 583.2 cm^{-1}; UV (absolute ethanol), 250.0, 305.6, 314.8, and 354.0 nm; mass, 225.03 (M+1)].

Treatment of cells. Cell lines were grown in RPMI 1640 medium supplemented with 20% fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin, at 37° in humidified incubators with 5% CO_2. PBMCs, isolated from buffy coats with a Ficoll density gradient, were washed three times in PBS and were

grown in the aforementioned medium supplemented with 1 μg/ml PHA-P. After 24-hr exposure to PHA-P, the supernatant was replaced with medium supplemented with 5% IL-2 (without PHA-P). Oltipraz and metabolite III were dissolved in DMSO and were stored at −70°. The stabilities of oltipraz and metabolite III in DMSO were assessed by high performance liquid chromatography using a Microsorb C_{18} column (Ranin, Emeryville, CA) run under isocratic elution conditions (methanol/water, 80:20), and the antiretroviral activities of old stocks were routinely compared with those of freshly prepared stocks at the time that the old stocks were replaced. There was neither chemical nor biological evidence for degradation of either drug in DMSO stored at −70° for >1 year (data not shown). The DMSO stocks were diluted into medium, to 0.2% maximal final concentration, just before treatment of the cells. Because the cells were cultured with 20% fetal bovine serum, no obvious precipitation of oltipraz or metabolite III was observed with final concentrations up to 200 μM.

Infection and assay of HIV-1 replication in H9 and CEM cells. Antiviral activity was assessed in H9 and CEM cells as described (9). Briefly, H9 and CEM cells were infected with HIV-1 (isolate HTLV-IIIB) at 1000 50% tissue culture infectious doses/10^6 cells. Virus was allowed to adsorb for 1 hr, after which the unadsorbed virus was removed by centrifugation. After the cells were washed twice with PBS, they were added to 96-well microtiter plates with medium containing up to 0.2% DMSO and various concentrations of oltipraz or metabolite III, at a final density of 500,000 cells/ml (final volume/well, 200 μl). On day 3 or 4, 50% of each supernatant was discarded and replaced with freshly prepared medium containing the same drug concentration as that in which the cells were plated initially. On day 7, cell densities were estimated by staining with MTT (10) and HIV-1 replication was assessed by measurement of p24 antigen in culture supernatants (Coulter, Hialeah, FL) or by indirect immunofluorescence, as described (9).

Assay of HIV-1 replication in PMA-stimulated U1 and ACH-2 cells. U1 and ACH-2 cells were treated as described (10), by incubating the cells (50,000 cells/microtiter well, 200-μl final volume) with various concentrations of oltipraz or metabolite III for 24 hr before the addition of 2.5 nM PMA. The cells were grown for an additional 48 hr, and p24 antigen release was measured in the culture supernatants.

Infection and assay of HIV-1 replication in PBMCs. PBMCs were used for experiments 2–6 days after PHA-P stimulation. Except for two experiments, PBMCs for each experiment were obtained from different donors. The PBMCs were treated as described for H9/CEM cell lines, except that the cells were plated at a density of 10^6 cells/ml in medium supplemented with 5% IL-2.

Cytotoxicity assays. Uninfected PBMCs, H9 cells, and CEM cells were treated as described above for infected cells. On day 7, cell densities were estimated by MTT staining (10). For U1 and ACH-2 cells, the cytotoxicity of oltipraz and metabolite III was assessed by MTT staining of matched non-PMA-stimulated cells.

Assay for inhibition of RT. Isotopic RT assays were performed as described (16), except that 4 μM (final concentration) unlabeled dTTP was added to all incubation mixtures. Assays were performed in duplicate or triplicate.

Statistical treatment of results. All data presented for H9, CEM, and U1 cells and PMBCs are expressed as percentage inhibition of HIV-1 or cell growth, relative to matched controls. For each experiment, the effects of treatment with various drug concentrations were assessed in duplicate sets of wells, whereas two to four wells of cells were used for controls. To adequately present the results of multiple independent experiments without bias, the individual experiments were treated as independent points and the average ± standard error values of the percentage inhibition for all experiments were determined. The ED_{50} (concentration for 50% inhibition of viral replication), IC_{50} (concentration for 50% reduction in cell density), and synergy estimates from pooled or individual experiments were determined by the median-effect principle, as described (17). Two independent experiments were performed with ACH-2 cells, wherein all drug concentrations and controls were assayed in triplicate sets of wells. The data presented are results from one of these experiments and are representative of the other. Statistical significance was analyzed by unpaired t test; $p < 0.05$ was taken as a statistically significant difference from PMA-stimulated controls.

Results and Discussion

Metabolite III was synthesized in 91% yield according to the method of Fleury *et al.* (13). The synthesis of metabolite III from cysteine demonstrates that oltipraz is an electrophile. The electrophilicity of oltipraz may be relevant to its biological properties, because oltipraz is thought to inactivate and/or modify proteins that are critical to its antischistosomal (i.e., schistosomal glutathione *S*-transferases) (18), anticarcinogenic (i.e., transcription factors) (19, 20), and antiviral (i.e., RT) (9) activities. We tested metabolite III as an antiviral agent because it is the major metabolic intermediate of oltipraz *in vivo* and our results with PMA-stimulated U1 cells suggested that oltipraz may be a prodrug in this chronic infection model (10).

In acutely infected H9 and CEM cells, metabolite III treatment resulted in a concentration-dependent inhibition of p24 antigen release into culture supernatants (Fig. 2). For both cell lines, the ED_{50} was approximately 25 μM. Oltipraz was approximately 2.5-fold more potent than metabolite III. Oltipraz and metabolite III inhibited viral replication in H9

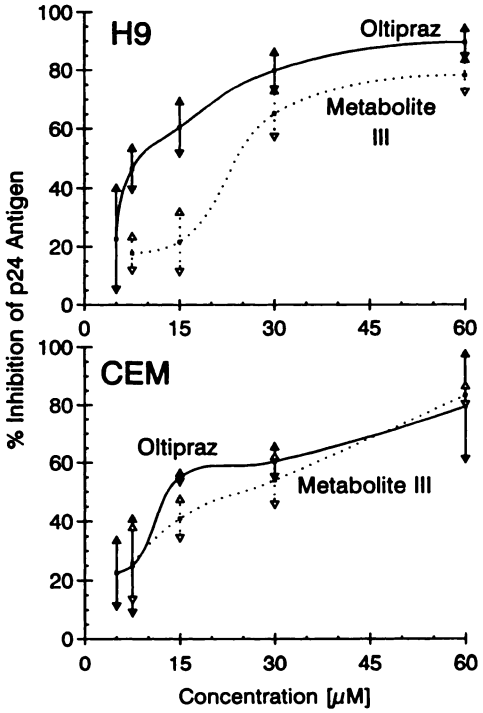

Fig. 2. Inhibition of p24 antigen release into culture supernatants of H9 (*upper*) and CEM (*lower*) cell lines as a function of the concentration of metabolite III or oltipraz. The inhibition of p24 antigen release was determined by normalizing p24 antigen levels to those of matched, HIV-1-infected (drug-free), control cells. Culture conditions and assays were performed as described in Experimental Procedures. *Symbols*, mean ± standard error of the percentage inhibition of p24 antigen release for seven and five independent experiments for the H9 and CEM cell lines, respectively. The IC_{50} values for zidovudine were determined to be 0.050 and 0.010 μM for the H9 and CEM cell lines, respectively.

cells with similar potencies, using indirect immunofluorescence as a surrogate marker for viral replication (data not shown). Although our assays examining the antiviral activity of metabolite III and oltipraz were not performed above 60 μM, cytotoxicity studies in uninfected cells indicated that metabolite III was relatively nontoxic (Table 1). The selectivity index (IC_{50}/ED_{50}) for metabolite III was 6.6 in H9 cells and 4.4 in CEM cells.

The inactivation of RT by oltipraz is a proposed mechanism for the antiretroviral activity of the drug (9) and, as noted above, it is likely that oltipraz acts as an electrophile by covalently modifying a nucleophilic amino acid residue of HIV-1 RT. In stark contrast, metabolite III had negligible effects on purified recombinant HIV-1 RT when assayed for its ability to reversibly or irreversibly inhibit the enzyme (Fig. 3). The inability of metabolite III to inactivate RT is not surprising, because metabolite III is not an electrophile. Moreover, given the hypothesis that the antiparasitic and anticarcinogenic activities of oltipraz are related to the ability of oltipraz to modify target proteins, the ineffectiveness of metabolite III as an antischistosomal agent and as an inducer of phase II enzymes would be expected (20).

The antiviral effects of metabolite III in the setting of chronic infection were examined because the drug clearly possessed antiviral activity but had no effect on RT. We were interested in determining whether metabolite III could block a point in the viral life cycle that was distal to viral integration, perhaps explaining our results with U1 cells (10). The ability of oltipraz and metabolite III to inhibit PMA-stimulated HIV-1 replication in chronically infected U1 (promonocytic leukemia) and ACH-2 (T cell lymphoma) cell lines was examined. In U1 cells, oltipraz (10) and metabolite III (40.4 ± 6.5% inhibition of HIV-1 replication at 60 μM, six independent experiments) inhibited PMA-induced HIV-1 replication. In ACH-2 cells, metabolite III was an effective inhibitor of PMA-induced HIV-1 replication, whereas oltipraz was inactive (Fig. 4). Metabolite III did not exhibit any cytotoxicity in either cell line at up to 60 μM.

Our results with ACH-2 cells were particularly useful for narrowing the potential mechanisms by which oltipraz and metabolite III inhibit HIV-1 replication. Although oltipraz was active as an inhibitor of HIV-1 replication in acutely infected H9 and CEM T cell lymphoma models (Fig. 2), oltipraz was devoid of antiviral activity in chronically infected ACH-2 (T cell lymphoma) cells (Fig. 4). Although it is possible

TABLE 1

Cytotoxicity of oltipraz and metabolite III in H9 cells, CEM cells, and PBMCs

Cell densities were determined by MTT staining in uninfected cells cultured for 7 days in medium containing 0–200 μM oltipraz or metabolite III, as described in Experimental Procedures. The IC_{50} for each independent experiment was determined by the median-effect principle (17). The results shown are the mean ± standard error of independent IC_{50} values.

Drug	IC_{50}		
	H9 cells	CEM cells	PBMCs
	μM		
Oltipraz	>200 (n = 1)[a,b]	>200 (n = 1)[a]	55.6 ± 5.6 (n = 6)
Metabolite III	166 ± 13 (n = 3)	109 ± 3 (n = 3)	54.8 ± 8.7 (n = 5)

[a] No cytotoxicity at concentrations up to 60 μM in multiple independent experiments.

[b] n, number of independent experiments. All experimental points were determined with two to four wells.

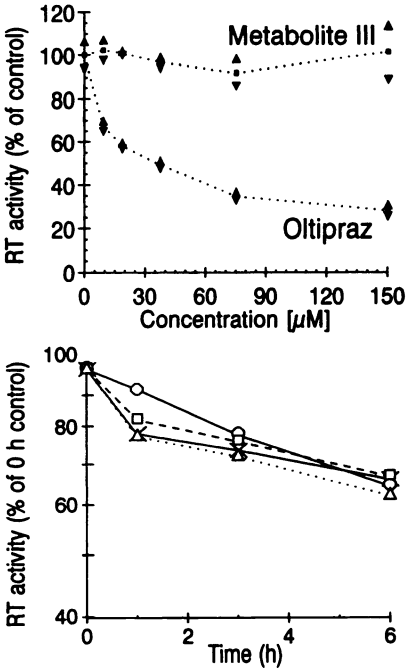

Fig. 3. Effects of oltipraz and metabolite III on RT activity as a function of concentration (*upper*) and the effect of metabolite III as an irreversible inhibitor of RT (*lower*). *Upper*, 10-μl aliquots of PBS containing RT, 1 mg/ml bovine serum albumin, 3.5% Triton X-100, and 0–525 μM metabolite III or Oltipraz were added to 25 μl of RT reaction buffer [50 mM Tris·HCl, 75 mM KCl, 5 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM EGTA, 0.1% Nonidet P-40, 0.1% Triton X-100, 10 μg/ml poly(A)$^+$ RNA, 2.5 μg/ml oligo(dT), 4 μM dTTP, 20 μCi/ml [α-^{32}P]dTTP, pH 7.8] in wells of a 96-well microtiter plate. After the mixtures were incubated for 0.5 hr at 37°, the remainder of the isotopic assay was performed as described by Chavan and Prochaska (16). *Symbols*, mean ± standard error for the enzyme assay. *Lower*, RT dissolved in PBS containing 1 mg/ml bovine serum albumin was preincubated with 56 (×), 28 (△), 14 (□), or 0 (○) μM metabolite III for the times indicated. After incubation, 10-μl aliquots (in triplicate) were removed and assayed for RT activity as described above.

that differences in the uptake and metabolism of oltipraz are responsible for the inactivity of oltipraz in ACH-2 cells, that is less likely than an intrinsic difference in the mechanism of action of oltipraz and metabolite III, because ACH-2 cells are ultimately derived from the CEM cell line. The ability to inhibit acute, but not chronic, replication in these T cell models supports our proposal that oltipraz inhibits HIV-1 replication via the inactivation of RT (9). In stark contrast, metabolite III is an effective inhibitor of HIV-1 replication in ACH-2 cells, suggesting that metabolite III acts to inhibit viral replication at a step distal to the integration of the viral genome. The findings in U1 cells may result from the ability of monocytoid cells to metabolize oltipraz to metabolite III and would account for the markedly increased potency of oltipraz with prolonged incubation before PMA stimulation (10). Whether oltipraz is metabolized differently in monocytoid versus T cell lines is an important question and will be investigated.

We assessed the ability of oltipraz and metabolite III to inhibit viral replication in acutely infected PMBCs (Fig. 5). Oltipraz was able to inhibit HIV-1 replication with an ED_{50} of 11 μM, whereas the ED_{50} for metabolite III was 18 μM (eight independent experiments). Interestingly, the dose-response curve for inhibition of viral replication by metabolite

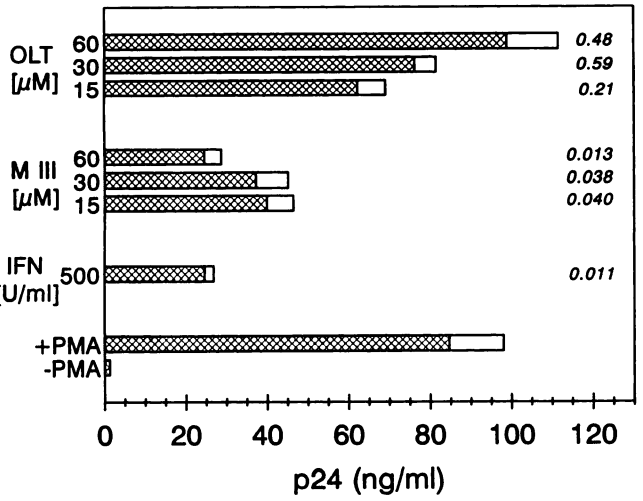

Fig. 4. Inhibition of p24 antigen release into culture supernatants from PMA-stimulated ACH-2 cells by metabolite III (*M III*) and interferon-α (*IFN*) but not oltipraz (*OLT*). ACH-2 cells (50,000 cells/microtiter well, 200-μl final volume) were incubated with various concentrations of oltipraz or metabolite III for 24 hr before the addition of 2.5 nM PMA, in triplicate sets of wells. The cells were grown for an additional 48 hr, and p24 antigen release into culture supernatants was determined. Shown are the means (■), standard errors (□), and *p* values (*numbers on the right*); *p* values of <0.05 were considered significantly different from PMA-stimulated controls.

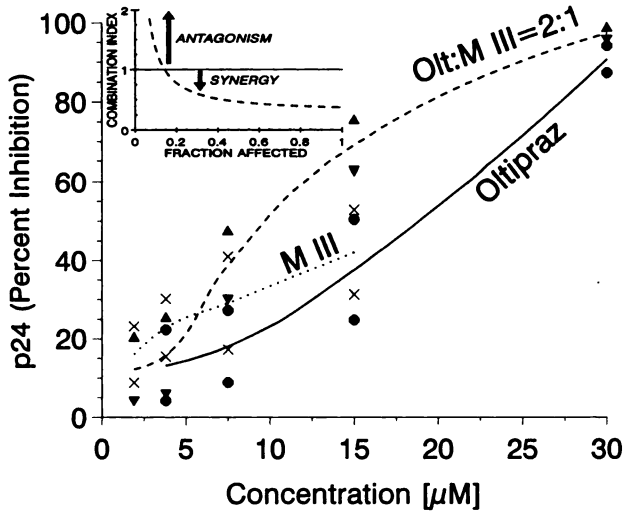

Fig. 5. Antiviral activity of oltipraz (●), metabolite III (*M III*) (×), and the combination of oltipraz/metabolite III (*Olt:M III*) at a fixed ratio of 2:1 (▲, ▼) on acutely infected PBMCs. PHA-P-stimulated lymphocytes were prepared and treated with oltipraz and/or metabolite III and virus as described in Experimental Procedures. *Symbols*, mean ± standard error for five independent experiments (wherein the combination of oltipraz and metabolite III was tested). *Inset*, corresponding combination index as a function of fractional inhibition of p24 antigen release. The median-effect plots [−log [(1/fractional inhibition) − 1] as a function of log (concentration); see Ref. 17 for derivation] yielded the following parameters, which were used to generate the combination index plot shown (*inset*): oltipraz, ED_{50} = 13.0 μM, slope = 1.94, and r = 0.925; metabolite III, ED_{50} = 27.1 μM, slope = 0.626, and r = 0.994; oltipraz/metabolite III (2:1), ED_{50} = 7.31 μM, slope = 1.94, and r = 0.953.

III was much shallower than that for oltipraz. It was also clear that the inhibitory activity of metabolite III varied more markedly than did that of oltipraz. For example, in three of eight independent experiments the ED_{50} for metabolite III was <5 μM. Whether this reflects differences in donor lymphocytes and/or the time at which stimulated PBMCs were exposed to metabolite III and HIV-1 after PHA-P treatment is at present unknown and is under investigation. It might be expected that agents acting at a site distal to integration of the viral genome would be more susceptible to differences in donor lymphocytes and/or the time of culture. The cytotoxicities of oltipraz and metabolite III in PBMCs are given in Table 1. The selectivity indices for oltipraz and metabolite III in PBMCs were 5- and 3-fold, respectively.

Because metabolite III is formed from oltipraz *in vivo*, we were interested in determining whether they were synergistic. The dose-response curves for oltipraz, metabolite III, and oltipraz/metabolite III (2:1) are shown in Fig. 5, and the combination indices calculated from these data are shown in Fig. 5, *inset*. Oltipraz and metabolite III were synergistic at fractional inhibitions of >15% (a combination index of <1 indicates synergy).

The observation that oltipraz and metabolite III are synergistic inhibitors of HIV-1 replication raises questions regarding the potential *in vivo* activity of oltipraz, especially because most of the absorbed drug undergoes rearrangement to metabolite III (11, 12). Significantly, rearranged metabolites related to metabolite III represent >75% of ^{14}C-labeled oltipraz recovered from the serum or urine (11, 12). Moreover, the serum concentration of oltipraz at nontoxic doses in humans and rodents has been documented to be in the low micromolar range (up to 20 μM) (3–8). Thus, oltipraz and its rearranged metabolites are found in serum at concentrations that inhibit HIV-1 replication *in vitro*, and our results suggest that oltipraz and its metabolites could act to synergistically inhibit HIV-1 replication *in vivo*.

In conclusion, the results presented here demonstrate that metabolite III inhibits HIV-1 replication *in vitro*. This is the first reported biological activity for this compound. In T cells, metabolite III appears to be an inhibitor of HIV-1 replication at a point in the replicative life cycle that is distal to viral integration. Therefore, just as oltipraz represents a new lead for the design of inhibitors of acute replication (and RT), metabolite III represents a new lead compound for the prevention of HIV-1 replication in chronically infected cells. Oltipraz and metabolite III inhibit HIV-1 replication via different mechanisms, and our results with PBMCs demonstrate that oltipraz and metabolite III are synergistic. Thus, oltipraz may be a more effective antiretroviral agent *in vivo* than it is *in vitro*. Because of this possibility, a forthcoming clinical trial evaluating the ability of oltipraz to reduce viral load will include an examination of oltipraz and metabolite III pharmacokinetics.

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Send reprint requests to: Hans J. Prochaska, Laboratory for Chemical-Biological Interactions, Molecular Pharmacology and Therapeutics Program, 601-E Rockefeller Research Laboratories, Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY 10021.