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# Drug Interactions with Zidovudine Phosphorylation In Vitro

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**We have investigated the effect of a range of drugs (some commonly coadministered with zidovudine [ZDV] to human immunodeficiency virus-positive patients) on intracellular phosphorylation of ZDV by stimulated peripheral blood mononuclear cells, Molt 4 cells, and U937 cells in vitro. Of the drugs tested (azoles, antiviral agents, antibiotics, and anticancer agents), only doxorubicin and ribavirin caused inhibition of anabolite formation as measured by high-performance liquid chromatography. This in vitro approach may provide important leads to potential interactions at the phosphorylation level in patients with human immunodeficiency virus disease. It is reassuring that so many commonly administered drugs do not alter ZDV phosphorylation.**

Zidovudine (ZDV), in common with other nucleoside analogs, requires intracellular phosphorylation via ZDV monophosphate (ZDV-MP) and ZDV diphosphate (ZDV-DP) to the active form ZDV triphosphate (ZDV-TP) which competitively inhibits viral reverse transcriptase and results in proviral DNA chain termination (1, 13, 19, 21, 23, 26).

Pharmacokinetic drug-drug interactions involving ZDV may occur at various stages, including absorption, hepatic metabolism (to glucuronide conjugate), and renal excretion of both ZDV and ZDV-glucuronide. Numerous studies have been conducted in these areas and have been recently reviewed (5, 18). However, since intracellular phosphorylation is a key event in the antiretroviral action of ZDV, it is important to design appropriate studies to investigate factors that may modify phosphorylation and hence drug efficacy. In a previous study (28) we demonstrated that didanosine and zalcitabine had no effect on ZDV phosphorylation in vitro in peripheral blood mononuclear cells (PBMCs). In the present work we describe the effect of a number of drugs (antiviral agents, antibiotics, antifungal agents, and others) regularly coadministered with ZDV to human immunodeficiency virus-positive patients on in vitro phosphorylation in activated PBMCs, Molt 4 cells, and U937 cells. A decrease in phosphorylation may have implications for in vivo efficacy of the nucleoside.

RPMI medium was purchased from Gibco, Life Technologies Ltd. Penicillin-streptomycin and L-glutamine were obtained from Northumbria Biologicals. Fetal calf serum was acquired from Sera Lab, Sussex, United Kingdom. Mono-poly resolving medium and ribavirin were obtained from ICN Biomedicals Inc., Irvine, Calif. Doxorubicin was obtained from Farmitalia Carlo Erba Ltd., St. Albans, Herts, United Kingdom. Acyclovir, ganciclovir, foscarnet, fluconazole, and ketoconazole and itraconazole were gifts from Wellcome Research Laboratories, Syntex Pharmaceuticals Ltd., Astra Pharmaceuticals, Pfizer Central Research, and Janssen Research Foundation, respectively. All other drugs and chemicals were purchased from Sigma Chemical Co. Ltd.

PBMCs were isolated from venous blood obtained from healthy volunteers as previously described (28) and were seeded in 5-cm-diameter petri dishes ($3 \times 10^6$ cells per plate). The mitogen phytohemagglutinin (10 μg/ml) was added, and the total volume was made up to 4 ml with RPMI growth medium. Cells were cultured at 37°C in a humidified 5% $CO_2$-gassed incubator for 72 h. Stimulated PBMCs were incubated with [$^3$H]ZDV (0.65 μCi; 0.018 μM) and either acyclovir, ganciclovir, foscarnet, ribavirin, doxorubicin, ketoconazole, fluconazole, itraconazole, erythromycin, rifampin, trimethoprim, sulfamethoxazole, 3′-amino-3′-dideoxythymidine (AMT), or thymidine (0.2 to 20 μM) for 5 h at 37°C in a humidified 5% $CO_2$-gassed incubator. Cell collection and extraction were as previously described (28). Samples were stored at −20°C until high-performance liquid chromatography (HPLC) analysis.

Molt 4 cells are a T-lymphoblastoid cell line; U937 cells are derived from monocytes. Cells were routinely maintained in 75-cm$^2$ flasks containing RPMI medium supplemented with 10% fetal calf serum and L-glutamine (2 mM) at 37°C in a humidified, 5% $CO_2$-gassed incubator. Cells ($4 \times 10^6$) were incubated with [$^3$H]ZDV (0.65 μCi; 0.018 μM) and a test drug (thymidine, AMT, ribavirin, or doxorubicin, 0.2 to 20 μM; others, 20 μM) for 3 h at 37°C in a humidified, 5% $CO_2$-gassed incubator. Experiments were performed in triplicate in six-well multiplates. Following incubation, cell suspensions were transferred to 7-ml silanized tubes, centrifuged, washed, recentrifuged, and extracted as described earlier for stimulated PBMCs. Quantitation of ZDV and ZDV phosphates by HPLC has been described in detail elsewhere (28).

Cell viability at the termination of an interaction study was assessed by the methods of trypan blue exclusion and neutral red uptake. In the neutral red assay, the dye (50 μg/ml) was added for a period of 3 h. Cells were harvested as described above and then washed twice with phosphate-buffered saline (500 μl). Destaining solution (1% glacial acetic acid, 50% ethanol; 500 μl) was then added to release the dye taken up by the cells. The $A_{540}$s were read. In addition, doxorubicin (20 μM) was incubated with all cell lines for 24 h (neutral red was added after 21 h) to produce cytotoxicity and then allow observation of the effect on neutral red uptake.

Previous studies have shown that optimal conditions for intracellular ZDV phosphorylation in PBMCs occur following a 72-h preincubation with the mitogen phytohemagglutinin at a concentration of 10 μg/ml (28). Under these conditions ZDV

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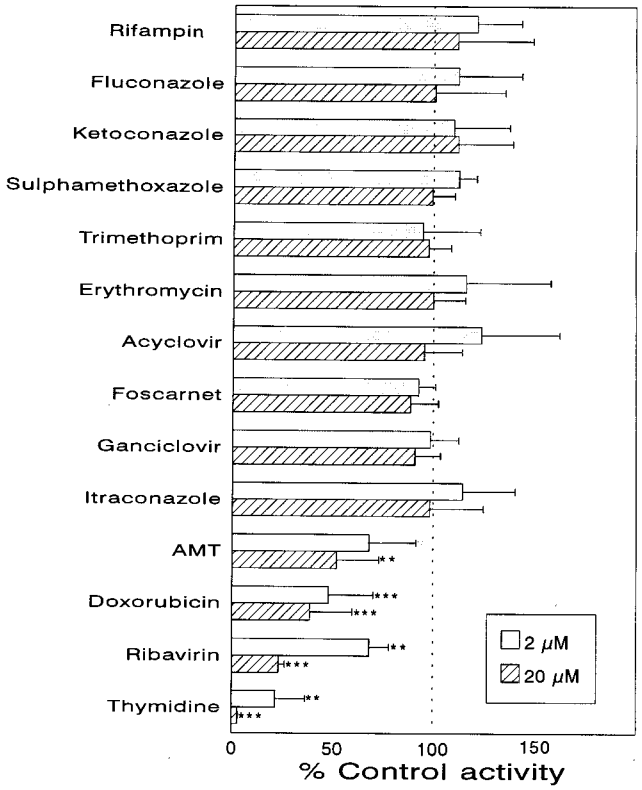


FIG. 1. Effects of various compounds on total ZDV phosphorylation in PBMCs. Each bar represents the mean ± standard deviation ($n \geq 5$). Data were analyzed by analysis of variance and a modified $t$ test (Bonferroni; paired comparisons). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (compared with control values).

(incubated for 5 h) was metabolized predominantly to the MP (approximately 75% of total phosphates) with lesser formation of the DP and TP. There was considerable variability in phosphorylation reflecting differential mitogen stimulation of PBMCs.

Thymidine inhibited formation of each phosphate anabolite at concentrations of 2 μM (30% of control total phosphate formation; $P < 0.05$) and 20 μM (3% of control phosphate formation; $P < 0.001$; Fig. 1). The antiviral agent ribavirin also significantly reduced ZDV phosphorylation at 2 and 20 μM (Fig. 1). At the highest concentration, total phosphorylation was reduced to 25% of the control value ($P < 0.001$). Similarly, doxorubicin significantly reduced formation of each phosphate anabolite. At the lowest concentration (0.2 μM) the MP was reduced ($P < 0.05$), and at the highest concentration (20 μM) total phosphate formation was 39% of the control level ($P < 0.001$; Fig. 1). Acyclovir, ganciclovir, foscarnet, ketoconazole, itraconazole, fluconazole, erythromycin, rifampin, trimethoprim, and sulfamethoxazole had no inhibitory effect on phosphorylation (Fig. 1). The only other compound inhibiting phosphorylation was the reduced metabolite of ZDV, AMT. None of the compounds inhibiting ZDV phosphorylation in PBMCs had any effect on cell viability over the 5-h incubation, as judged by trypan blue uptake, although with 20 μM doxorubicin there was a 20% decrease in neutral red uptake. However, there was a marked cytotoxic effect of doxorubicin when the incubation time was increased to 24 h (80% decrease in neutral red uptake).

In order to substantiate the data obtained in PBMCs, further studies were carried out with Molt 4 and U937 cells. In the Molt 4 cell line, there was increased turnover to the DP and TP anabolites of ZDV in comparison with that in PBMCs and U937 cells. The ratios of ZDV-TP to ZDV-MP were 0.17 (PBMCs), 0.30 (Molt 4 cells), and 0.04 (U937 cells). The results of coincubations with a range of drugs were consistent with the findings in PBMCs. Again both ribavirin and doxorubicin produce marked inhibition of phosphorylation, i.e., decreased anabolite formation, without any evidence of cytotoxicity. AMT also significantly reduced formation of each anabolite in both cell lines. None of the other compounds at a concentration of 20 μM produced any marked inhibition of phosphorylation (<15% inhibition).

TABLE 1. Drug interactions with ZDV phosphorylation in PBMCs, Molt 4 cells, and U937 cells

| Drug | Mean % inhibition of ZDV phosphorylation[a] | | | Peak concn (μM) in plasma (reference) |
|---|---|---|---|---|
| | PBMCs | Molt 4 cells | U937 cells | |
| Ribavirin | 76 | 90 | 45 | 15 (17) |
| Doxorubicin | 61 | 80 | 76 | 1.5 (10) |
| AMT | 53 | 42 | 23 | 0.7 (24) |
| Itraconazole | 13 | 0 | 0 | 1.1 (22) |
| Ganciclovir | 12 | 15 | 0 | 20 (12) |
| Foscarnet | 11 | 10 | 10 | 600 (27) |
| Acyclovir | 7 | 9 | 11 | 15 (30) |
| Erythromycin | 3 | 0 | 0 | 7 (15) |
| Trimethoprim | 0 | 10 | 0 | 6 (16) |
| Sulfamethoxazole | 0 | 0 | 0 | 230 (16) |
| Ketoconazole | 0 | 0 | 0 | 18 (4) |
| Fluconazole | 0 | 12 | 10 | 30 (25) |
| Rifampin | 0 | 0 | 0 | 12 (6) |

[a] Drug concentration of 20 μM ($n \geq 5$).

Although drug interactions with ZDV can occur at various stages after oral administration (5), inhibition of phosphorylation in blood mononuclear cells has obvious implications in relation to efficacy.

The predominance of the MP in stimulated PBMCs, Molt 4 cells, and U937 cells is believed to be the result of its relatively poor affinity for thymidylate kinase, the enzyme responsible for conversion of ZDV-MP to ZDV-DP. The concentrations of potentially interacting drugs ranged from 0.2 to 20 μM in an attempt to approximate to concentrations in plasma (Table 1; note that foscarnet and sulfamethoxazole concentrations are very much higher). We also used thymidine as a positive control since this compound is a substrate for thymidine kinase and therefore inhibits ZDV phosphorylation (26, 28). The other nondrug studied was AMT, a metabolite of ZDV (8, 9, 24) which has been suggested to impair the antiretroviral effect of ZDV (8). The data presented here indicate that AMT can reduce ZDV phosphorylation, although whether the relatively low concentrations found in vivo would be sufficient to alter ZDV anabolite formation is not clear.

The majority of drugs tested had no inhibitory effects on phosphorylation; this included the antiviral agents acyclovir and ganciclovir. The ganciclovir data differ from the findings of Gowland et al. (14), who demonstrated a dose-dependent reduction in intracellular ZDV phosphorylation. Both ganciclovir and acyclovir are phosphorylated, initially to the MP metabolite by viral thymidine kinase. However, it has been shown that in uninfected cells neither compound is an efficient substrate for mammalian thymidine kinase (7, 11). The antifungal and antimicrobial drugs were screened because of their mechanism of action involving enzyme inhibition and their widespread use in immunocompromised patients. It is reassuring to

see that these drugs did not produce any inhibition of ZDV phosphorylation.

However, ribavirin and doxorubicin significantly reduced ZDV phosphorylation in all cells. Ribavirin has been shown previously to antagonize the phosphorylation of pyrimidine dideoxynucleosides (2), and the mechanism is thought to involve a ribavirin-induced increase in nucleoside TP levels leading to feedback inhibition of thymidine kinase (29) and hence conversion of ZDV to ZDV-MP (20). As doxorubicin exerts its pharmacological effect by insertion between DNA base pairs, it is not clear how it alters ZDV phosphorylation. However, assessment of cell viability as measured by trypan blue exclusion, neutral red uptake, and cell counts before and after incubation suggests that the effect of doxorubicin is not simply cytotoxicity. The exact mechanism awaits further study.

In conclusion, we have used three in vitro cell systems to screen for possible drug interactions with ZDV phosphorylation. Of the therapeutic compounds tested, only doxorubicin and ribavirin inhibited phosphorylation. This approach may provide important leads to potential interactions at the phosphorylation level in patients with human immunodeficiency virus disease (3). Since the antiviral effect of ZDV is dependent on the ratio of ZDV-TP to thymidine-TP, any decrease in ZDV anabolite formation is likely to alter efficacy only if this ratio is markedly changed.

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