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The effect of thymidine on the antibacterial and antiviral activity of zidovudine

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Abstract—The effect of thymidine and deoxyadenosine on the antiviral and antibacterial effect of zidovudine was studied in human immunodeficiency virus type 1 (HIV-1) *Escherichia coli* and *Salmonella typhimurium*. In quantitative assays, 10 μg mL^{-1} thymidine was shown to increase the 50% inhibitory concentration (IC50) of zidovudine for HIV-1 by approximately 100-fold and to reduce zidovudine (1 μM)-induced protection of C8166 cells from 2·04 to 0·18 log syncytial-forming units. Thymidine also antagonized the antibacterial effect of zidovudine for two *E. coli* and three *S. typhimurium* species in a dose-dependent manner; 10 μg mL^{-1} of thymidine increased the minimum inhibitory concentration of zidovudine for *E. coli* strains by 10–40-fold and for *S. typhimurium* strains by three-fold. Deoxyadenosine reduced the minimum inhibitory concentration of zidovudine against all five bacterial strains but had no effect on the IC50 of zidovudine for HIV-1, nor did it significantly reverse the antagonism of the antibacterial and antiviral activity of thymidine. The induction of the SOS response in *E. coli* was reversed in a dose-dependent manner by thymidine while the presence of deoxyadenosine increased induction of the SOS response by zidovudine at suboptimal concentrations.

Zidovudine (3′azido-3′deoxythymidine) is a thymidine analogue that inhibits the replication of human immunodeficiency virus (HIV) in-vitro (Mitsuya et al 1985) and is beneficial in the treatment of patients with HIV infection (Fischl 1989). Zidovudine also displays antibacterial activity against the Enterobacteriaceae (Elwell et al 1987; Lewin & Amyes 1989). The antiviral properties of zidovudine are derived primarily from its ability to inhibit viral reverse transcriptase activity, while its antibacterial activity is caused by its action as a chain terminator during its incorporation into viral and bacterial DNA, the azido group making the formation of subsequent 5′–3′ phosphodiester linkages impossible (Mitsuya & Broder 1987; Hirsch 1988). Thymidine is incorporated into DNA by the same pathway as zidovudine and it is likely that phosphorylation of both is catalysed by the same nucleoside kinase (Furman et al 1986). It has been shown that thymidine suppresses zidovudine phosphorylation (Eriksson et al 1989; Szebeni et al 1991) and counteracts the antiviral effect of zidovudine (Mitsuya et al 1985; Sommadosi et al 1988). The purpose of this study was to determine the effect of thymidine and deoxyadenosine on the antibacterial activity of zidovudine against *Escherichia coli* and *Salmonella typhimurium* and to compare this quantitatively with their activities against HIV. In addition, since Mamber et al (1990) showed that zidovudine induced the SOS response (bacterial gap filling repair if damaged DNA) in *E. coli* and suggested that bacterial assays for the SOS response may be useful as screening assays for potential anti-HIV agents, it seemed appropriate to determine whether thymidine antagonized the role of zidovudine in induction of the SOS response.

Materials and methods

Drugs. Zidovudine was obtained from Wellcome (Beckenham, UK); thymidine and deoxyadenosine were obtained from Sigma Chemical Co. Ltd (Poole, UK).

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Virus assay. Cell cultures were maintained at 37°C/5% CO_2 in RPMI 1640 medium (Gibco, Paisley, UK) supplemented with 10% foetal bovine serum, L-glutamine, sodium pyruvate and antibiotics. Viral assays were performed in 96-well tissue culture plates using C8166 (Sodroski et al 1984) or MT4 (Harada et al 1985) cells and the HTLV-IIIB (Popovic et al 1984) strain of HIV-1. To assess the effect of thymidine and deoxyadenosine on the 50% inhibitory concentration (IC50) of zidovudine, C8166 or MT4 cells (3×10^4 per well) were suspended in quadruplicate in varying concentrations of zidovudine alone or with thymidine and deoxyadenosine and 100 tissue culture infectious doses (TCID) of virus added per well. The plates were incubated for 6 days, after which time cytopathic effect was determined visually. IC50 values were calculated by the Karber formula (Karber 1931).

To determine the effect of thymidine on zidovudine-induced protection in terms of infectious units of HIV-1, C8166 cells were suspended in 96-well plates (3×10^4 cells per well) in varying concentrations of zidovudine alone or with thymidine in a total volume of 150 μL. To each well was added 100 μL of the appropriate dilution of HIV-1 stock virus serially diluted from $1:10^{-1}$ to $1:10^{-6}$. The plates were then incubated for 3–4 days as above and syncytia were counted. Titres, initially expressed as mean (n = 4) syncytial-forming units per well were converted into logarithmic values and protection indices calculated by subtraction of titres from control titrations performed in the absence of zidovudine and thymidine.

Bacterial strains. E. coli AB1157, *E. coli* GC4415 (Huisman & D'Ari 1981), *E. coli* KL16, *S. typhimurium* M329 and *S. typhimurium* NCTC5710 were used in this study. The strains were grown on nutrient agar plates which were subcultured every 10 days.

Minimum inhibitory concentrations. Minimum bacterial inhibitory concentrations (MICs) of zidovudine alone or in the presence of thymidine or deoxyadenosine, were determined on Diagnostic Sensitivity Test Agar (Oxoid, UK) using an arithmetic dilution scheme described by Smith (1984). An inoculum of 10^4 colony forming units was placed on the plates using a multipoint inoculator (Denley, UK). The plates were incubated at 37°C overnight and the MIC defined as the lowest concentration which inhibited growth.

Induction of the SOS response. The induction of the SOS response in *E. coli* GC4415, a strain possessing a lacz-sfiA fusion (Huisman & D'Ari 1981), by zidovudine alone and in the presence of thymidine or deoxyadenosine was measured by a colorimetric assay as described by Miller (1972) with the exception that Isosensitest broth was substituted for LB broth. SOS induction was expressed as β-galactosidase activity in Miller units derived from the formula:

$$\frac{1000 \times OD_{420} - 1{\cdot}75 \times OD_{550}}{t \times v \times OD_{600}}$$

where OD_{420} and OD_{550} were the optical densities at the relevant wavelength after t min; v was the reaction volume and OD_{600} was the initial optical density at 600 nm.

Table 1. The effect of varying concentrations of thymidine on the antiviral effect of zidovudine. IC50 values and protection indices (log syncytial forming units) were calculated as described in Materials and methods.

		Zidovudine				
Thymidine ($\mu g\ mL^{-1}$)	IC50 (μM)	Concn (μM) 0	0·01	0·1	1	10
0	0·04	—	0·46	0·96	2·04	2·22
0·1	0·04	0·26	0·44	0·70	1·51	2·12
1	0·3	0·04	0·14	0·14	1·04	2·04
10	4	0·14	0·20	0·04	1·18	1·04
100	20	0·21	0·34	0·25	0·27	0·27

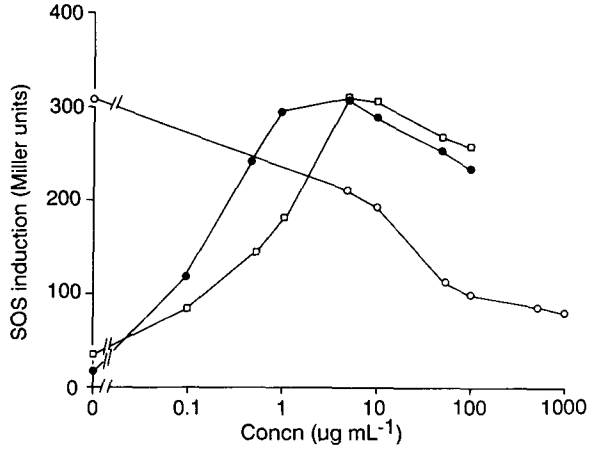

FIG. 1. Induction of the SOS response in *E. coli* GC4415 exposed to zidovudine alone (□), zidovudine plus deoxyadenosine (200 $\mu g\ mL^{-1}$ ●), and zidovudine (5 $\mu g\ mL^{-1}$) plus thymidine (○). SOS induction was expressed as β-galactosidase activity in Miller units as described in Materials and methods.

Results and discussion

Mitsuya et al (1985) first showed that thymidine antagonized the anti-HIV-1 activity of zidovudine in a dose-dependent manner. In the present study we have extended these observations using sensitive quantitative assays in C8166 and MT4 cells. The effects of varying concentrations of thymidine on the anti-HIV activity of zidovudine are presented in Table 1. The 50% inhibitory concentration of zidovudine for 100 TCID of virus in C8166 cells was 0·04 μM, which increased approximately 10-fold to 0·3 μM in the presence of as little as 1 $\mu g\ mL^{-1}$ thymidine, rising progressively to 20 μM in the presence of 100 $\mu g\ mL^{-1}$ thymidine. Identical results were observed in MT4 cells (data not shown). Similarly, increasing concentrations of thymidine antagonized the zidovudine-induced protection of C8166 cells from HIV-1 in a dose-dependent manner; 10 μM zidovudine alone reduced the titre of HIV-1 by 2·04 log units but in the presence of 10 $\mu g\ mL^{-1}$ thymidine reduction was insignificant (0·18 log units). The effects of varying concentrations of thymidine or deoxyadenosine on the MIC of zidovudine for five bacterial species are shown in Table 2. The presence of thymidine antagonized the antibacterial effect of zidovudine against all five strains in a dose-dependent manner although the activity of zidovudine against the *E. coli* strains was more susceptible to antagonism than its activity against *S. typhimurium*. This phenomenon may be related to the approximately 10-fold lower sensitivity of *S. typhimurium* strains to zidovudine compared with *E. coli* strains.

Deoxyadenosine stimulates thymidine phosphorylase activity and therefore increases the efficiency of incorporation of thymidine into DNA. It seemed probable that deoxyadenosine would also increase the efficiency of incorporation of zidovudine into DNA, thus increasing its antibacterial or antiviral activity. Indeed deoxyadenosine (50 or 200 $\mu g\ mL^{-1}$) caused a 3–5-fold reduction in the MIC of zidovudine against all five bacterial strains tested (Table 1). However, the presence of deoxyadenosine (50 $\mu g\ mL^{-1}$) in the medium did not reduce the IC50 of zidovudine for HIV-1 in MT4 cells (data not shown). In addition, the presence of 50 $\mu g\ mL^{-1}$ deoxyadenosine did not significantly reverse the antagonism of the antibacterial (Table 1) or anti-HIV-1 (data not shown) activity of zidovudine by thymidine. The effect of thymidine and deoxyadenosine on the induction of the SOS response by zidovudine was determined for *E. coli* CC4415. Thymidine at concentrations from 5–1000 $\mu g\ mL^{-1}$ reduced the induction of the SOS response by zidovudine 5 $\mu g\ mL^{-1}$, the concentration at which peak induction occurred (Fig. 1). The induction of the SOS response by zidovudine decreased as the thymidine concentration rose confirming that thymidine reduces the DNA damage caused by zidovudine. In contrast, the presence of deoxyadenosine increased induction of the SOS response by zidovudine at sub-optimal concentrations, supporting the results of the MIC assays which showed that this compound can potentiate the antibacterial activity of zidovudine.

In conclusion, the results of this study indicate that the presence of low levels of thymidine will adversely affect the antibacterial and antiviral properties of zidovudine in-vitro. It is therefore essential that MIC and IC50 determinations, or assays for induction of the SOS response, should be carried out in media containing a well defined minimal amount of thymidine to ensure reproducibility of results. Furthermore, whether this phenomenon extends to other nucleoside analogues with potential antiviral or antibacterial properties such as dideoxyadenosine dideoxyinosine (Mamber et al 1990) should be investigated.

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Table 2. MIC values ($\mu g\ mL^{-1}$) of zidovudine for three *E. coli* and two *S. typhimurium* strains in the presence of varying concentrations of thymidine and deoxyadenosine (DA).

	Thymidine ($\mu g\ mL^{-1}$)							DA ($\mu g\ mL^{-1}$)		Thymidine/DA ($\mu g\ mL^{-1}$)
Strain	0	1	5	10	25	50	100	50	100	50/50
E. coli (AB1157)	0·04	0·75	1	1·5	2	2	2	0·01	0·01	1
E. coli (GC4415)	0·04	0·03	0·3	0·4	2	2	2	0·01	0·01	1
E. coli (KL16)	0·08	0·05	0·4	0·75	2	2	5	0·01	0·01	1
S. typhimurium (M329)	0·5	0·75	1·5	1·5	7·5	7·5	10	0·2	0·2	5
S. typhimurium (NCTC5701)	0·5	0·75	1·5	1·5	7·5	7·5	10	0·2	0·2	5

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