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Susceptibility of *Treponema pallidum* and other selected spirochaetes to zidovudine

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The antiviral nucleoside derivative zidovudine (3′-azido-3′-deoxythymidine) previously has been shown to be an effective antibacterial agent in animals infected with *Escherichia coli* or *Salmonella typhimurium*. Since HIV infection can alter the course of human syphilis with serious consequences, it was of interest to determine if the noncultivable spirochaetal agent of syphilis, *Treponema pallidum*, is susceptible to this compound. The progression of experimental rabbit syphilis over a three week period was unchanged in animals receiving either 50 or 150 mg/kg oral zidovudine daily. In addition, a number of cultivable pathogenic and nonpathogenic spirochaetes were tested for susceptibility to zidovudine *in vitro*. At a concentration of 100 mg/L, zidovudine had no detectable effect on spirochaete growth, morphology, or motility. Thus it appears that spirochaetes are generally not susceptible to this compound, and that long-term zidovudine therapy will not be of benefit in preventing or controlling syphilis or other spirochaetoses in HIV-infected humans receiving this drug.

Introduction

The synthetic nucleoside zidovudine (3′-azido-3′-deoxythymidine) is presently the most effective licensed antiviral agent for the treatment of human immunodeficiency virus (HIV) infections (Mitsuya *et al.*, 1985; Fischl *et al.*, 1987). Interestingly, many Gram-negative bacteria, particularly enteric bacteria, are extremely sensitive to zidovudine *in vitro* with MICs of less than 0·5 mg/L (Elwell *et al.*, 1987; Lewin & Amyes, 1989). Zidovudine, however, exhibits little or no activity against other bacteria, including *Pseudomonas aeruginosa, Mycobacterium tuberculosis,* various anaerobes and all Gram-positive bacteria (Elwell *et al.*, 1987; Lewin & Amyes, 1989). There is no information concerning the susceptibility of spirochaetes to zidovudine.

In-vivo studies using the *Salmonella dublin* calf oral infection model and the *Escherichia coli* mouse systemic infection and ascending pyelonephritis models found zidovudine to be highly efficacious (Keith, White & Wilson, 1989). A recent review of the rate of relapses of non-typhoid salmonella bacteraemia in HIV-infected patients suggests that zidovudine therapy prevents further relapses, thus obviating antibiotic therapy (Salmon *et al.*, 1991). Humans co-infected with HIV and the syphilis spirochaete, *Treponema pallidum*, can manifest unusual, often severe exacerbation of syphi-

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litic symptoms (Johns, Tierney & Felsenstein, 1987; Radolf & Kaplan, 1988), and syphilis in these patients appears somewhat refractory to conventional benzathine penicillin G therapy (Berry *et al.*, 1987; Lukehart *et al.*, 1988). Since many HIV-infected individuals are maintained on long-term zidovudine therapy, it was of interest to determine the efficacy of this compound against *T. pallidum* and certain other species of spirochaetes.

Material and methods

Animals

Adult male New Zealand white rabbits (3–4 kg), obtained from a local supplier, were fed antibiotic-free food and were housed at 18°–20°C prior to and following infection with *T. pallidum*. Only rabbits that were serologically nonreactive for infection with *Treponema paraluiscuniculi*, as assessed by the Venereal Disease Research Laboratory (VDRL) slide flocculation test (Fisher Scientific, Pittsburgh PA, USA) and the micro-haemagglutination assay for *T. pallidum* antibodies (MHA-TP) (Ames Laboratories, Elkhart IN, USA) were used in this study. All protocols involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill.

Bacterial strains

The source of *T. pallidum* subspecies *pallidum* Nichols strain, cultivation of the organism in rabbit testes, and enumeration of the freshly extracted treponemes has been previously described (Miller, 1971; Stamm *et al.*, 1983). The sources of the cultivable spirochaetes, *Treponema phagedenis* and *Treponema denticola* (Stamm *et al.*, 1983; Stamm & Bassford, 1985) and *Leptospira biflexa* and *Leptospira interrogans* (Nunes-Edwards *et al.*, 1985) have also been previously described. *Borrelia burgdorferi* strains were obtained from Alan Barbour, San Antonio TX, USA. *E. coli* ATCC 23355 and *P. aeruginosa* ATCC 27853 were obtained from the American Type Culture Collection (Rockville MD, USA).

Zidovudine treatment of rabbits and intradermal challenge with T. pallidum

A group of three New Zealand white rabbits was used for each of the two doses of zidovudine tested (50 and 150 mg/kg). An additional untreated rabbit was included in each group as a positive control. Zidovudine was provided by Burroughs Wellcome Co., Research Triangle Park NC, USA. The drug was adminstered two days before intradermal challenge with *T. pallidum* and was continued for three weeks postchallenge. Each rabbit received its total daily dose of zidovudine in distilled H_2O in two feedings 6 h apart via gastric lavage. The animals did not require sedation during the administration of the drug. Blood samples for determination of serum zidovudine levels (determined by high performance liquid chromatography at Burroughs Wellcome Co. as described by Good, Reynolds & de Miranda (1988), and for serology were withdrawn from the marginal ear vein of each rabbit 45 min following the second daily dose of zidovudine. Rabbits were anaesthetized by intramuscular injection of each animal with 300 mg ketamine hydrochloride in combination with 3·0 mg acepromazine maleate (Aveco, Ft. Dodge IA, USA). Each rabbit was injected intradermally on the clipped back with 0·1 mL of a suspension of *T. pallidum* containing 4×10^3 cells (at two

anterior sites) and 4×10^4 cells (at two posterior sites). The backs of all the rabbits were clipped regularly and the injection sites were observed on a daily basis for development of characteristic syphilitic lesions (chancres).

In-vitro assays for susceptibility of cultivable spirochaetes to 100 mg/L zidovudine

T. phagedenis biotype Reiter bacteria grown in Spirolate broth (BBL Microbiology Systems, Cockeysville MD, USA) supplemented with 10% heat-inactivated normal rabbit serum (HI-NRS) supplied by JRH Biosciences, Lenexa KS, USA were diluted in the same medium with or without zidovudine to give $6{\cdot}7 \times 10^6$ cells/mL. *T. denticola* strain 11 was grown in PPLO broth (BBL Microbiology Systems) supplemented with 10% HI-NRS and 5 mg/L cocarboxylase (Jacob, Allen & Nauman, 1979) and diluted in the same medium with or without zidovudine to give 2×10^7 cells/mL. Cultures were incubated under anaerobic conditions at 37°C and monitored for growth over a five day period. Cells of *L. interrogans* serovar *hardjo*, a human leptospirosis isolate, and *L. biflexa* serovar patoc, a saprophytic leptospire, grown in bovine serum albumin-polysorbate 80 medium (Ellinghausen & McCullough, 1965) were diluted in the same medium with or without zidovudine to give 2×10^7 cells/mL. Cultures were incubated at 30°C and monitored for growth with a photonephelometer (Johnson & Harris, 1967). Cells of *B. burgdorferi* strains HB19 and B31, the Lyme disease agent, grown in BSKII medium containing 5% HI-NRS (Barbour, 1984) were diluted in the same medium with or without zidovudine to give 8×10^6 cells/mL. Cultures were incubated at 30°C and monitored for growth over a four day period. A 10 μL sample from each culture was examined at the end of the incubation periods by dark-field microscopy (Nikon Optiphot, Tokyo, Japan) at 600 × total magnification to observe cell morphology and motility. The in-vitro susceptibility of control organisms (*E. coli* and *P. aeruginosa*) to zidovudine at concentrations 10 and 100 mg/L was determined as previously described (Lewin & Amyes, 1989) except that Luria broth was substituted for nutrient broth.

Results

Due to the inability to continuously culture *T. pallidum in vitro*, we used the rabbit model to determine the in-vivo susceptibility of this spirochaete to zidovudine. As shown in Table I, the group of rabbits receiving 50 mg/kg zidovudine daily was determined to have serum levels ranging from 1·34–2·85 mg/L. The group receiving the higher 150 mg/kg zidovudine daily dose had serum levels ranging from 12·45–14·98 mg/L. These levels fall within the range expected for each dose in rabbits (Dr K. Ayers, personal communication). The lower than proportional level of zidovu-

Table I. Susceptibility of *T. pallidum* to zidovudine *in vivo*

Zidovudine dose (mg/kg/day)	Serum levels (mg zidovudine/L)[a]	No. of rabbits infected/symptomatic	MHA-TP titres
0	NA NA	2/2	≥ 1:2560
50	1·78, 1·34, 2·85	3/3	≥ 1:2560
150	12·45, 14·18, 14·98	3/3	≥ 1:2560

[a]Determined by HPLC (Good *et al.*, 1988).

Table II. Susceptibility of cultivable spirochaetes to zidovudine *in vitro*

Bacterium	Growth medium	Growth in presence of 100 mg/L zidovudine
T. phagedenis (*Reiter*)	Spirolate broth[a]	+
T. denticola (strain 11)	PPLO broth[b]	+
L. biflexa (*patoc*)	BSA polysorbate 80	+
L. interrogans (*hardjo*)	BSA polysorbate 80	+
B. burgdorferi (HB19, B31)	BSK II[c]	+

[a]Supplemented with 10% heat-inactivated rabbit serum and grown under anaerobic conditions.
[b]Supplemented with 10% heat-inactivated rabbit serum and 5 mg/L cocarboxylase and grown under anaerobic conditions.
[c]Supplemented with 5% heat-inactivated rabbit serum.

dine found in the rabbits in the 50 mg/kg dose group was probably because these animals achieved their peak serum levels at an earlier time. For the higher dose cohort, the level of drug determined for serum drawn 45 min following the second daily administration of zidovudine was probably close to the peak level (Dr P. de Miranda, personal communication).

Syphilitic lesions first appeared as small maculopapules in both the zidovudine treated and untreated control rabbits at approximately seven to nine days following ID challenge with *T. pallidum*. Within a three week period, the lesions at all of the inoculation sites of the rabbits from both zidovudine treated groups progressed to raised, erythematous, indurated nodules with haemorrhagic, necrotic centres. The appearance of the lesions and the rate of development in these animals were identical to those of the untreated control animals (data not shown). Serological confirmation of syphilitic infection was quantitatively determined by the MHA-TP test in accordance with the manufacturer's instructions (Shore, 1974; Tight & White, 1980). Serum MHA-TP titres in all rabbits reached 1 in 2560 at approximately three weeks postchallenge (Table I) which correlates positively with the potential for infective lymph node transfer (unpublished data). Identical results were obtained with a duplicate group of rabbits receiving 150 mg/kg zidovudine daily (data not shown). Based on these results, there is no indication that zidovudine therapy at either dose prevented, delayed, or exacerbated the normal symptomatic progression of primary syphilis in the rabbit.

The results of the in-vitro susceptibility of selected cultivable spirochaetes to zidovudine are presented in Table II. There was no detectable difference in the increase in turbidity of cultures containing 100 mg/L zidovudine versus control cultures for any of the spirochaetes tested. The cell morphology and motility of the organisms grown in the presence of zidovudine appeared no different to those of cells from control cultures lacking zidovudine. *E. coli* and *P. aeruginosa* were used as control organisms for the in-vitro susceptibility testing. As expected, *E. coli* cells were susceptible to zidovudine at 10 and 100 mg/L after a 4 h exposure, whereas *P. aerginosa* cells were resistant to both concentrations of zidovudine (data not shown).

Discussion

Since early experimental syphilitic infection in the rabbit resembles the primary stage of human syphilis, the rabbit model has been extensively used for evaluation of various

prophylactic or therapeutic regimens (Turner & Hollander, 1957; Lukehart & Baker-Zander, 1987; Johnson, 1989). We have used this model to determine the susceptibility of animals treated with zidovudine to infection with *T. pallidum* following intra-dermal challenge. Our results indicated that continuous zidovudine therapy had no effect on the progression of experimental rabbit syphilis. During the course of chancre development, serum levels of zidovudine in the rabbits appeared adequate for antibacterial action against susceptible organisms. The serum levels of zidovudine attained in the higher dose group were within the same range as those found to be efficacious in the calf salmonellosis and mouse *E. coli* infection studies (Keith *et al.*, 1989). Furthermore, the serum zidovudine levels in our animals were significantly higher than those measured in the plasma of normal humans receiving zidovudine intravenously or orally at doses higher than that currently recommended for HIV-infected patients (Klecker *et al.*, 1987). Rabbits metabolize zidovudine to the inactive glucuronide form more slowly than do humans (Dr P. de Miranda, personal communication).

The lack of in-vivo susceptibility of *T. pallidum* to zidovudine prompted us to determine whether certain cultivable spirochaetal species were also resistant to this compound. Since members of the order Spirochaetales are genetically and metabolically diverse, we could not predict *a priori* the response of these organisms to zidovudine. In-vitro susceptibility testing of two pathogenic spirochaetes, *B. burgdorferi* and *L. interrogans*, as well as three nonpathogenic spirochaetes, indicated that these organisms were not susceptible to zidovudine at a concentration that was several times the concentration found to be active against certain Gram-negative enteric bacteria.

Elwell *et al.* (1987) have shown that zidovudine must be metabolically activated to the nucleotide level by thymidine kinase in order to inhibit cellular metabolism. Organisms that lack thymidine kinase activity (e.g. *Mycobacterium tuberculosis*, *M. intracellulare*, and *Pseudomonas aeruginosa*) are not susceptible to zidovudine (Elwell *et al.*, 1987; Saito & Tomioka, 1984). Additionally, spontaneous mutants of *E. coli* or *S. typhimurium* that are resistant to zidovudine are also deficient in thymidine kinase activity. The inability of zidovudine to prevent experimental syphilitic infection of rabbits and to inhibit the in-vitro growth of cultivable spirochaetes appears consistent with the lack of information concerning pyrimidine metabolism in these organisms. Both *T. pallidum* and *T. phagedenis* cells were reported to lack detectable thymidine kinase activity and were incapable of incorporating [^{3}H]thymidine into nucleic acids (Baseman, Nichols & Mogerly, 1979; Norris, Miller & Sykes, 1980). *L. interrogans* also failed to incorporate radiolabelled pyrimidines into their nucleic acids (Johnson & Rogers, 1964). An absence of thymidine kinase activity could thus readily explain the resistance of spirochaetes to zidovudine. However, in the case of *T. pallidum* it should be noted that these organisms are obtained from infected rabbits in very limited quantities; thus, the results of enzyme assays wth treponemal extracts are not uniformly reliable. For example, the gene encoding 1-pyrroline-5-carboxylate reductase, the terminal enzyme in the proline biosynthetic pathway, was recently identified from a *T. pallidum* genomic library, despite the fact that the enzyme was undetectable in extracts of treponemes (Gherardini *et al.*, 1990). Recent attempts to identify a gene encoding thymidine kinase in a *T. pallidum* genomic library were unsuccessful (unpublished data), further indicating that this organism likely lacks the enzyme. Our data regarding the lack of susceptibility of spirochaetes to zidovudine indicates that long-term zidovudine therapy of HIV-infected humans will not be

beneficial in the prevention or control of syphilis or other spirochaetoses in these patients.

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