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In Vivo Efficacy of Zidovudine (3′-Azido-3′-Deoxythymidine) in Experimental Gram-Negative-Bacterial Infections

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The therapeutic efficacy of orally administered zidovudine (3′-azido-3′-deoxythymidine) was determined in animals infected with *Escherichia coli* and *Salmonella dublin*. The 50% effective dose (ED_{50}) of zidovudine (9.6 to 11.8 mg/kg of body weight) compared favorably with that of trimethoprim (19.4 to 22.2 mg/kg) in mice with systemic *E. coli* infection. At 50 mg/kg, both zidovudine and ampicillin reduced the number of bacteria in the kidneys of mice and prevented lethal infection in mice with ascending pyelonephritis caused by *E. coli*. Zidovudine prevented a lethal *S. dublin* infection in calves over a wide dose range (8.0 to 31.0 mg/kg per day). Zidovudine levels in plasma of uninfected mice were 28.2 ± 4.5 and 7.9 ± 2.2 μg/ml at 30 and 60 min, respectively, exceeding the MICs for the bacteria used in the infections. Few zidovudine-resistant strains were observed. The in vivo data raise the possibility that zidovudine may have an antibacterial effect in patients receiving this therapy.

The synthetic nucleoside zidovudine (3′-azido-3′-deoxythymidine) is an antimicrobial agent with a spectrum of in vitro activity that encompasses human immunodeficiency virus (HIV) (8), the protozoan *Giardia lamblia* (S. Nusinoff-Lehrman, M. St. Clair, R. L. Miller, S. Broder, H. R. Wilson, M. Bushby, et al., Program Abstr. Int. Conf. AIDS, abstr. no. 556, 1985), and gram-negative bacteria, including *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, and *Haemophilus influenzae* (1). Zidovudine has shown no activity against *Pseudomonas aeruginosa*, gram-positive or anaerobic bacteria, or common pathogenic fungi (such as *Pneumocystis carinii*) frequently isolated from HIV-infected patients (Nusinoff-Lehrman et al., Int. Conf. AIDS, 1985). *E. coli* and *Salmonella dublin* have been shown to be exquisitely susceptible to zidovudine, with in vitro MICs in the range of 0.0025 to 0.3 μg/ml (Table 1).

We extended these in vitro observations to determine the in vivo efficacy of zidovudine in experimental bacterial infections that included a mouse systemic *E. coli* infection, acute ascending *E. coli* pyelonephritis in mice, and *S. dublin* salmonellosis in calves.

MATERIALS AND METHODS

Antimicrobial agents. Zidovudine and trimethoprim were laboratory reference preparations (Burroughs Wellcome Co., Research Triangle Park, N.C.). Ampicillin was purchased as the sodium salt (Sigma Chemical Co., St. Louis, Mo.). For all studies on mice, compounds were dissolved or suspended just before use in an aqueous suspending vehicle consisting of 1.0% Tween 80 and 0.5% low-viscosity sodium carboxymethylcellulose (Sigma). For studies in calves, zidovudine was administered by subcutaneous injection in an aqeuous solution of 20% dimethylformamide.

Bacteria. The bacteria used for animal infections were veterinary and clinical isolates previously found to exhibit susceptibility in vitro to zidovudine and the reference antimicrobial agents used in this study. *E. coli* P855, used for the mouse systemic infection studies, is a clone from *E. coli* CN348 (isolated from a sick calf) maintained in the Wellcome Research Laboratories strain collection. *E. coli* J96 was used for the mouse ascending pyelonephritis studies and is a human pyelonephritis isolate (3). *S. dublin* M738, used for the calf salmonellosis studies, was isolated from a sick calf (16; Table 1).

Animals. Mice were obtained from Charles River Breeding Laboratories, North Wilmington, Mass. CD-1 female mice 5 to 6 weeks old (19 to 21 g) were used for systemic *E. coli* infections; BALB/c female mice 6 to 7 weeks old (19 to 20 g) were used for the pyelonephritis model. Friesian calves for systemic *S. dublin* studies were purchased at about 1 week of age (40 to 45 kg) in a market in southwest England.

Systemic *E. coli* infections. Mouse-passaged *E. coli* P855 was grown to late exponential phase in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) at 30°C with vigorous shaking, diluted 10-fold in a mixture of brain heart infusion broth and 15% glycerol, and cryopreserved in vapor-phase storage over liquid nitrogen. Samples of the frozen bacteria were thawed and diluted in sterile buffered saline plus gelatin (NaCl, 8.5 mg/ml; anhydrous KH_2PO_4, 0.3 mg/ml; anhydrous Na_2HPO_4, 0.6 mg/ml; gelatin, 0.1 mg/ml) for each test. CD-1 mice were inoculated by intraperitoneal injection of 100 50% lethal doses (approximately 2×10^5 CFU) of cryopreserved *E. coli* P855 in a suspension of 0.2% agar (Bacto-Agar; Difco) containing 1% iron dextran (Imferon without phenol as preservative; Merrell Dow Pharmaceutical Inc., Cincinnati, Ohio), equivalent to 12.5 mg of ferric iron per kg of body weight.

Zidovudine, trimethoprim, or aqueous suspending vehicle was administered orally at 25, 12.5, 6.2, and 3.1 mg/kg to randomized groups of 10 mice each (0.2 ml/20 g of body weight) at 1 and 4 h after infection. No animals died after 4 days. The number of animals surviving for 4 days after infection was used to calculate the 50% effective dose (ED_{50}).

***E. coli* pyelonephritis.** The ascending *E. coli* pyelonephritis model has been previously described (10). Briefly, BALB/c mice were inoculated with *E. coli* J96, a clinical urinary tract isolate that possesses both the mannose-sensitive and mannose-resistant pili that are implicated as virulence factors in urinary tract infections (14). Simultaneous expression of the

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TABLE 1. Susceptibilities and descriptions of bacterial strains used in zidovudine efficacy studies in vivo

Strain[a]	Model	MIC (μg/ml)			Remarks
		Zidovudine	Trimethoprim	Ampicillin	
E. coli					
P855	Mouse systemic infection	0.1	0.1	ND[b]	Clone derived from *E. coli* CN348 (isolated from sick calf) from Wellcome Research Laboratories strain collection
J96	Mouse ascending pyelonephritis	0.1	ND	3.0	Human pyelonephritis isolate; motile, hemolytic, colicin V positive, simultaneously expresses Gal-Gal, mannose-resistant pili (3)
S. dublin M738	Calf salmonellosis	0.3	ND	ND	Isolated from sick calf (16)

[a] Frequency of spontaneously arising mutants resistant to 10 μg of zidovudine per kg estimated in vitro to be 4.4×10^{-7} for *E. coli* P855, 1.8×10^{-7} for *E. coli* J96 (L. Elwell, personal communication), and 10^{-7} for *S. dublin* M738.
[b] ND, Not determined.

mannose-sensitive and -resistant pili by *E. coli* J96 was accomplished by single-colony isolation on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) from the kidneys of a previously infected mouse, followed by static culture for 18 h on Trypticase soy broth (BBL) at 37°C. The presence of mannose-sensitive and -resistant pili was confirmed by slide agglutination of guinea pig mannose-sensitive (pili) and human blood group P (mannose-resistant pili) erythrocytes (10, 11). The culture was then diluted 20-fold in sterile Trypticase soy broth for inoculation. Mice were anesthetized with ether. A catheter (PE 10 polyethylene tubing; Becton Dickinson and Co., Parsippany, N.J.) was inserted transurethrally to a depth of 1.5 cm, and 0.2 ml of inoculum (approximately 10^7 CFU) was instilled into the bladder. Mice were then randomized to cages (five mice per cage) and allowed to recover without further manipulation.

The therapeutic efficacy of zidovudine was compared with that of ampicillin, which is routinely used in the treatment of human pyelonephritis infections (12). Previous studies by us (data not shown) indicated that 50 mg/kg is the minimum effective dose for both zidovudine and ampicillin in this model. Therefore, individual mice were randomized to treatment groups of 9 to 10 each and were given 50 mg of zidovudine, ampicillin, or aqueous suspending vehicle per kg by oral administration (0.2 ml/20 g of body weight) at 1, 3, and 5 h after infection. Mice were killed 24 h after infection by halothane (Ayerst Laboratories, New York, N.Y.) inhalation overdose. Pairs of kidneys were aseptically excised and homogenized in 10 ml of buffered saline with gelatin, using a tissue grinder (Bio-Homogenizer; Biospec Products Inc., Bartlesville, Okla.). Serial 10-fold dilutions of kidney homogenates were made in buffered saline with gelatin, and 0.1 ml of each was spread on Trypticase soy agar plates for CFU counts. The incidence of zidovudine-resistant *E. coli* was determined by spreading 0.1 ml of the undiluted kidney homogenates on Trypticase soy agar plates containing 50 μg of zidovudine per ml.

Zidovudine in normal mouse plasma was assayed by high-pressure liquid chromatography (S. S. Good and D. J. Reynolds, Program Abstr. 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 377, 1987). Zidovudine was dissolved in aqueous suspending vehicle, and a single dose of 50 mg/kg was administered orally to BALB/c mice. Mice were killed by ether inhalation overdose at 0.5 and 1.0 h; blood specimens were collected from vena cava puncture, with EDTA as the anticoagulant. Plasma was separated by centrifugation at 1,850 × *g* for 10 min at room temperature. Plasma specimens were diluted in normal mouse plasma (Sigma) for high-pressure liquid chromatography analysis.

Systemic *S. dublin* infection. The calf salmonellosis experiments were performed as previously described (15). Equal portions of an exponential-phase culture of *S. dublin* M738 were stored at −70°C in nutrient broth containing 10% glycerol. The frequency of zidovudine-resistant mutants in this stock was estimated by spreading 0.1 ml of thawed, cryopreserved portions on Iso-Sensitest agar (Oxoid Ltd., London, England) plates supplemented with 10 μg of zidovudine per ml. For preparation of inocula, 3 ml of culture stock was added to 200 ml of tryptone soya broth (Oxoid) and incubated with shaking at 37°C for 4 h. The culture was adjusted with a nephelometry standard to contain 2×10^8 CFU/ml. Calves that were previously found free of *Salmonella* carriage were infected at approximately 17 days of age. All food was withdrawn overnight before infection. An inoculum consisting of 50 ml (10^{10} CFU, total) of adjusted *S. dublin* culture was administered orally to each calf with an esophageal tube attached to a 60-ml syringe.

Two experiments were performed to assess the therapeutic efficacy of zidovudine. In both experiments, zidovudine was administered by subcutaneous injection. In a preliminary experiment, one calf per dose was treated once daily with 8, 16, or 31 mg of zidovudine per kg on days 2, 3, and 4 after infection. The susceptibility of posttreatment rectal isolates of *S. dublin* was determined by surface growth inhibition by 10 μg of zidovudine per ml incorporated in Iso-Sensitest agar. In the second experiment, three calves received single injections of 20 mg/kg on days 2, 3, and 4 after infection, and two calves similarly received injections at 10 mg/kg; five other infected calves remained untreated.

Concentrations of zidovudine in serum samples from uninfected calves were determined by bioassay, using a well-plate method with Iso-Sensitest agar supplemented with 4 μg of sulfadiazine per ml to enhance the susceptibility of the test strain, *E. coli* NCTC 10418. Zidovudine was dissolved in 20% dimethylformamide, and a single dose of either 20 or 10 mg/kg was administered subcutaneously to calves. Serum samples were obtained 5 h after administration of zidovudine.

Statistical analysis. ED_{50}s were calculated by probit analysis (6). Fisher's exact test was used to determine significance in the pyelonephritis experiments. In statistical analyses in which differences were tested, significance was declared when P was <0.05. The standardized method of infection with *S. dublin* M738 was shown to produce 86% mortality in 81 untreated calves used in a series of experiments (15); this total has since been increased to more than 100 calves, with consistent mortality. On this basis, the probability of occurrence of the mortality rates in the *S. dublin* experiments was calculated according to the binomial

TABLE 2. Efficacies of zidovudine and trimethoprim against systemic *E. coli* P855 infections in mice[a]

Study	Compound[b]	ED_{50}[c] (mg/kg)
1	Zidovudine	9.6 (6.9–13.4)
	Trimethoprim	22.2 (21.2–23.1)
2	Zidovudine	11.8 (11.3–12.4)
	Trimethoprim	19.4 (9.8–38.1)

[a] Number of 50% lethal doses, 100; number of CFU, 2×10^5 per mouse.
[b] Mice were dosed orally at 1 and 4 h after infection.
[c] Computed by probit analysis; numbers in parentheses are 95% confidence limits.

distribution (13), using an individual survival probability of 0.14.

RESULTS

Systemic *E. coli* infections. The therapeutic efficacies of zidovudine and trimethoprim were assessed in female CD-1 mice inoculated intraperitoneally with *E. coli* P855 (Table 2). Mortality among untreated mice approached 100% within 48 h after infection. In both tests, the ED_{50}s exhibited by zidovudine were approximately one-half of the trimethoprim ED_{50}s. In the first study, zidovudine was clearly more active than trimethoprim; in the second study, the large 95% confidence interval for trimethoprim prevented the ED_{50}s from being significantly different. Nevertheless, these data indicate that zidovudine is at least as effective as trimethoprim in this model.

Mouse pyelonephritis. Mice were infected transurethrally with *E. coli* J96 and treated orally at 1, 3, and 5 h after infection with 50 mg of zidovudine, ampicillin, or aqueous suspending vehicle per kg (Fig. 1). Three of ten (30%) of the untreated mice died within 24 h. Most (80%) of the untreated mice harbored a kidney bacterial load that exceeded 10^5 CFU/g of kidney tissue and had symptoms characterized by hematuria and hypoactivity. In contrast, no mice in the groups receiving zidovudine or ampicillin died or exhibited overt symptoms. For both the zidovudine and ampicillin treatment groups, the number of mice without severe infections posttreatment (less than 10^5 CFU/g of kidney tissue) was significantly lower than in the control group ($P < 0.05$). The only mouse in the zidovudine treatment group with a severe infection was found to harbor zidovudine-resistant *E. coli*. Also, the number of mice without detectable infections was significantly higher in the zidovudine treatment group than in the control group ($P < 0.05$); this was not the case for ampicillin ($P > 0.05$).

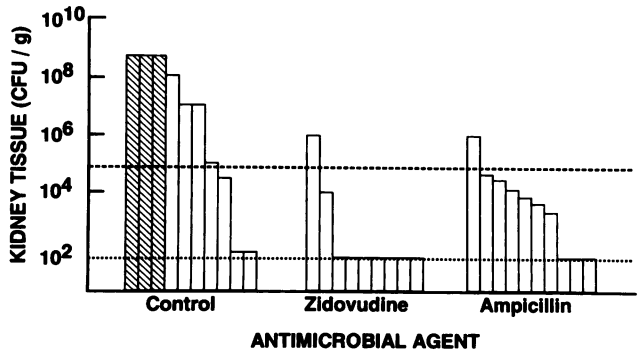

FIG. 1. Effects of oral treatment of zidovudine or ampicillin on acute ascending pyelonephritis produced by *E. coli* J96. Mice were given 50 mg/kg at 1, 3, and 5 h after infection. Each bar represents one animal. Mice dead 24 h after infection (▨) were assumed to have 5.0×10^8 CFU/g in the kidneys (2, 7). Kidney burdens of $\geq 10^5$ CFU/g (---) were considered severe; limit of detection of bacteria was 10^2 CFU/g (....).

To obtain levels of zidovudine in plasma, high-pressure liquid chromatography analysis was performed on plasma samples from normal mice treated with a single oral dose of 50 mg of zidovudine per kg. On the basis of susceptibility studies with *E. coli* J96, one goal of this trial was to determine whether levels in plasma that exceeded the MIC for *E. coli* J96 (0.1 μg/ml) could be attained. Indeed, at 30 and 60 min, mean zidovudine levels in plasma were 28.2 ± 4.5 and 7.9 ± 2.2 μg/ml, respectively; these values easily exceeded target levels.

Calf salmonellosis. The development of symptoms after infection followed the expected pattern; by the time of first treatment on day 2, all calves except one control in experiment 2 were exhibiting lassitude, pyrexia (>40°C), and severe diarrhea. The mortality pattern is shown in Table 3. Survival of one of the five untreated calves was consistent with the expected overall mortality of 86% in this well-established disease model ($P = 0.38$). In the preliminary experiment, all three treated calves survived. With an expected individual survival rate of $P = 0.14$, the chance of a group of three calves surviving would have been $P = 0.003$ in the absence of an effective treatment. In the second experiment, one of two calves given 10 mg/kg per day died, whereas all three calves given 20 mg/kg survived. The overall survival rate associated with zidovudine, independent of dose, was significant ($P < 0.05$) by Fisher's exact test.

For each experiment, decimal dilutions of an *S. dublin* inoculum plated on agar containing zidovudine (10 μg/ml) indicated that the proportion of resistant mutants was about 10^{-7} CFU/ml. In the first experiment, no *S. dublin* isolates resistant to zidovudine were obtained from rectal swabs of the treated calves. No attempt was made to isolate *S. dublin* from rectal swabs in the other experiment.

A bioassay was used to obtain levels of zidovudine in serum of uninfected calves (data not shown). Five hours after subcutaneous administration of zidovudine, the mean zidovudine levels in serum were 1.07 ± 0.33 μg/ml (20 mg/kg) and 0.36 ± 0.16 μg/ml (10 mg/kg), well above the MIC for *S. dublin* M738.

DISCUSSION

The data reported here show that zidovudine is effective in the treatment of experimental infections caused by the gram-negative bacteria *E. coli* and *S. dublin*. In the mouse systemic and pyelonephritis models, zidovudine was comparable with trimethoprim and ampicillin in therapeutic efficacy against *E. coli*. In its pathogenesis and pathology, the experimental disease induced by oral infection of calves closely resembles natural infection by *S. dublin* in this species (15). *S. dublin* bears a host-adapted relationship to cattle that is similar to the relationship of *Salmonella typhi* to humans, and the diseases these organisms cause are very similar, with bacteremia and progressive focal lesions in internal organs, particularly the liver. The good results in calves therefore suggest that zidovudine may also be effective against typhoid and other systemic *Salmonella* species infections in humans. In this and previous studies in experimental animals (S. S. Good, D. T. Durack, and P. de Miranda, Fed. Proc. **6f**:444, 1986), analysis of levels in

TABLE 3. Results of zidovudine treatment on systemic *S. dublin* M738 infections in calves

Study	Zidovudine dose[a] (mg/kg per day)	No. of calves		Remarks
		Infected	Survived[b]	
1	31	1	1	Symptoms became minimal by 24 h after first dose of zidovudine; calf fully recovered by day 5
	16	1	1	Symptoms diminished by day 5; calf recovered by day 9
	8	1	1	Symptoms diminished by day 5; calf recovered by day 9
2	Control	5	1	Four calves died on days 6, 7, 7, and 9; calf without symptoms on day 2 survived
	20	3	3	Symptoms diminished by day 5; calves fully recovered by day 9
	10	2	1	One calf died on day 7; symptoms diminished in other calf by day 5, with full recovery by day 9

[a] Calves were dosed subcutaneously once daily on days 2, 3, and 4 after infection.
[b] Survival, independent of dose, associated with zidovudine treatment; $P < 0.05$ (Fisher's exact test).

plasma and urine indicate that zidovudine is well absorbed after oral administration and is excreted primarily as parent compound, although the degree of biotransformation to the glucuronide metabolite varies among species.

In the experiments described here, zidovudine was administered as a single oral (mouse systemic and pyelonephritis infections) or subcutaneous (calf *S. dublin* infections) dose. Patients with human T-lymphotrophic virus type III infections receive more intensive therapy (typically, 200 mg of zidovudine administered orally every 4 h). In a pharmacokinetic study of zidovudine in normal individuals, levels of zidovudine in plasma were determined after a single oral dose of 5 mg/kg (4). At 30 and 60 min, levels in plasma were 1.34 and 0.67 μg/ml, respectively, similar to levels in blood reported here. The comparative half-lives of zidovudine in mice (0.5 h, single oral dose), calves (2.0 h, single subcutaneous dose), and humans (1.1 h, single oral dose; 4) suggest that the single-dose treatment schedule for these animal experiments was not optimal.

The emergence of zidovudine resistance has been demonstrated in vitro in selected strains of *E. coli* and *S. typhimurium* (1). The in vitro frequency of mutation to zidovudine-resistant *E. coli* is approximately 2×10^{-7} to 4×10^{-7} (Table 1). Zidovudine serves as a substrate for bacterial thymidine kinase and human T-lymphotrophic virus type III reverse transcriptase and is incorporated into the growing DNA chain, resulting in chain termination. Experiments performed by Elwell et al. (1) show that growth rates of zidovudine-resistant *E. coli* are unaffected by high levels of zidovudine, which suggests that resistance is due to a stable mutational event and is apparently due to a lack of significant levels of thymidine kinase. No cross-resistance to other antimicrobial agents has been seen with zidovudine. Despite the inocula of 10^{10} CFU that were used in the calf salmonellosis experiments, no clinical or microbiological zidovudine resistance was documented, although a low proportion of zidovudine-resistant *S. dublin* mutants could be isolated from the original seed culture used to prepare the inocula. In the mouse pyelonephritis model, zidovudine-resistant *E. coli* were isolated from the kidneys of one of nine treated animals, which indicated that zidovudine-resistant bacteria may emerge in vivo; however, it is not known whether the appearance of resistant *E. coli* was due to selection of resistant organisms in the inoculum (10^7 CFU per animal) or to spontaneous mutation during the course of infection.

Bacterial infections are known to occur in adults and children with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. In one study, nosocomial infections caused by gram-negative bacilli were found to occur late in the clinical course of patients with AIDS and resulted in a high fatality rate (17). Of significance to the data reported in our study, both gastrointestinal and urinary tract infections caused by gram-negative bacilli, including gastroenteritis caused by *S. typhimurium* and urinary tract infections caused by *E. coli*, have been found in patients with AIDS (5, 9, 17). However, the limitations imposed by these short-term animal studies prevent any definite conclusions regarding the antibacterial efficacy in HIV-infected individuals receiving long-term zidovudine therapy. Hematological toxicity, including neutropenia, in HIV-infected individuals receiving zidovudine has been shown; also, opportunistic infections continue to occur in some patients receiving zidovudine therapy (M. A. Fischl, J. Reese, L. Dearmas, G. Dickenson, and W. Parks, 27th ICAAC, abstr. no. 386, 1987; K. Rolston, S. Radentz, S. Rodriguez, P. Mansell, and G. P. Bodey, Program Abstr. 4th Int. Conf. AIDS, abstr. no. 7083, 1988). The in vivo data reported in this paper suggest that zidovudine may modify the course of infection or provide suppressive therapy in certain bacterial infections in patients receiving this agent as primary therapy for HIV infections.

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