NOTES

Evidence That High-Dosage Zidovudine at Time of Retrovirus Exposure Reduces Antiviral Efficacy

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The antiviral efficacy of prophylactic 3'-azido-3'-deoxythymidine (AZT) therapy administered by continuous infusion or intermittent injection was compared in pediatric cats infected with feline leukemia virus. A 4-week treatment regimen of AZT was initiated at -48, 8, or 96 h postinfection (p.i.). For AZT therapy begun at -48 h p.i., significant efficacy was attained when therapy was given by continuous infusion but not by intermittent injection. However, when AZT therapy was delayed until 96 h p.i., both continuous infusion and intermittent injection gave complete protection. The results suggest that intermittent AZT administration is less efficacious than continuous infusion. Higher peak AZT concentrations in plasma associated with intermittent injection compared with those associated with continuous infusion may be immunotoxic, thus reducing the drug-induced vaccine effect. Furthermore, AZT toxicity seemed to be restricted to a window of sensitivity close to the time of virus challenge because delaying the start of AZT therapy until 96 h p.i. was highly efficacious, regardless of the method of administration.

3'-Azido-3'-deoxythymidine (AZT; zidovudine), the most widely used antiviral drug therapy for the treatment of AIDS, blocks retrovirus infection by inhibiting human immunodeficiency virus type 1 (HIV-1) reverse transcription (35, 53) and by feedback inhibition of thymidine kinase (18, 19). Because it acts on a preintegration stage of the replication cycle, AZT has the potential to completely prevent retrovirus infection. However, in murine, feline, and simian animal models of AIDS, AZT therapy, initiated before or immediately after virus exposure, reduced the incidence of disease but did not uniformly prevent primary infection (2, 3, 14, 20, 21, 31–34, 36, 47, 59). In humans the failure of postexposure prophylactic AZT therapy to prevent HIV-1 infections has been reported in numerous uncontrolled anecdotal accounts (13, 27, 29, 30, 39, 61, 62), but until recently, no careful evaluations of the prophylactic efficacy of AZT therapy were available. However, the results from a recent retrospective case-controlled study suggested that post-exposure AZT therapy reduced the risk of HIV infection by 79% (7). In addition, interim results from AIDS Clinical Trial Group protocol 076 indicated that AZT administration to HIV-infected pregnant women reduced the risk of perinatal infection by approximately one-third (6), although this effect may be due to the reduction of maternal virus load rather than drug prophylaxis in the fetus (12). Together, these and other studies indicate that while AZT therapy is not a cure for AIDS, if given prophylactically it can reduce the risk of HIV infection, and as therapy, it reduces the virus load in AIDS patients (48) and prolongs survival (16).

Factors that might contribute to the less than complete blocking of lentivirus infection by prophylactic AZT therapy include the selection of AZT-resistant variants (28), inadequate intracellular AZT triphosphate concentrations (1, 37),

cell-to-cell transmission of HIV which is not susceptible to AZT inhibition (55), selection of lymphocyte subsets that are resistant to AZT toxicity and insensitive to the antiviral activity of AZT (9), and AZT toxicity to immune cells. The immunotoxic effects of AZT have been shown primarily in *in vitro* studies in which AZT suppressed the growth of hematopoietic progenitors (25, 52), mitogen (23, 38) and antigen (58) stimulation of human lymphocytes, the development of cytotoxic lymphocyte function (49), and lymphokine-activated killer cell activity (54). To what extent, if any, AZT suppresses immune function *in vivo* is largely unknown.

Recent studies with HIV-infected long-term nonprogressors and HIV-negative homosexual males or female prostitutes with high-risk behavior suggest that a strong HIV-specific cytotoxic T-lymphocyte (CTL) response is an important if not essential factor for restricting virus expression (22, 50). If high-dosage AZT retards the development of the HIV-specific CTL response, the consequence may be enhanced pathogenesis. In fact, low CTL responses correlated with increased disease progression in HIV-infected patients (22).

We and others have previously evaluated different AZT regimens for their antiviral efficacies in the feline leukemia virus (FeLV) model for AIDS (33, 59). Normally, adult cats exposed to FeLV develop long-term immunity which protects them from FeLV-mediated and opportunistic diseases. In contrast, young cats are highly susceptible to FeLV infection (24). However, if prophylactic AZT therapy is given to experimentally infected young cats, the primary virus infection is attenuated to the extent that protective antiviral immunity can be established (33). This drug-induced vaccine effect was originally described in a murine retrovirus model of AIDS (46, 47). Because both the feline and murine animal models show protective immune responses to retroviruses, they have the potential to serve as good indicators of *in vivo* drug-related immunotoxicity in association with infection.

In earlier work, we defined the time window after viral

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TABLE 1. Effect of AZT treatment on FeLV viremia when AZT was administered at various doses or times p.i.

Group	Administration regimen	AZT start time (h) ^a	AZT dosage (mg/kg/day) ^b	No. of animals	FeLV antigenemia ^c	% Efficacy	Statistical significance (P value) ^d
A	Continuous infusion	-48	60	7	1	86	0.0047
B	Intermittent injection	-48	60	6	3	50	0.182
C	Oral	-48	100	6	3	50	0.182
D	Continuous infusion without bolus	8	60	6	1	83	0.015
E	Continuous infusion with bolus	8	60 + bolus ^e	6	4	33	0.454
F	Continuous infusion	96	60	6	0	100	0.001
G	Intermittent injection	96	60	6	0	100	0.001
H	Control	NA ^f	0	7	7	0	NA

^a Time that AZT treatment began relative to virus inoculation.^b AZT was administered by continuous subcutaneous infusion at the indicated dose for 28 days.^c Number of cats with chronic FeLV antigenemia determined by antigen-capture ELISA for FeLV p27.^d P value was determined by the Fisher exact test.^e AZT bolus dose of 3.6 mg/kg given at 8 h p.i.^f NA, not applicable.

inoculation in which AZT therapy prevented chronic FeLV viremia in cats (33). A 4-week regimen of 60 mg of AZT per kg of body weight per day administered by continuous infusion protected cats from chronic FeLV viremia if therapy was begun within 96 h postinfection (p.i.) but did not protect the cats if treatment was delayed to 196 h p.i. (33). In parallel studies, continuous and intermittent modes of drug administration were compared. A serendipitous finding, which we report here, was that AZT administration by an intermittent regimen instead of by continuous infusion close to the time of primary virus exposure significantly reduced antiviral drug efficacy. However, if intermittent AZT therapy was delayed until 96 h p.i., the antiviral efficacy of AZT was retained.

For these studies AZT, obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, was dissolved in pyrogen-free normal saline adjusted to pH 7.4 with 1 M NaOH. Specific-pathogen-free (SPF) cats were obtained from the Ohio State University SPF cat breeding colony. Animal studies were performed in accordance with the University Laboratory Animal Care and Use Committee and the guidelines of the National Institutes of Health (38a). FeLV antigenemia in plasma was determined by a commercially available antigen-capture enzyme-linked immunosorbent assay (ELISA) for FeLV p27 protein (VIRACHEK FeLV ELISA kit; Synbiotics Corp., San Diego, Calif.). FeLV used as challenge virus consisted of pooled plasma from SPF cats infected with FeLV as described previously (33, 41, 57). The pooled plasma contained 4×10^3 focus-forming units per ml as determined by the S+L-81c cell assay (17). The 100% animal infectious dose for all cats in the study was 0.1 ml of infectious plasma injected intravenously. AZT was given to 8- to 10-week-old cats (33) beginning at -48, 8, or 96 h p.i., depending on the study protocol, and was continued for 4 weeks. Table 1 lists the different treatment and control animal groups. AZT was administered by continuous subcutaneous infusion (56) at a dosage of 60 mg/kg/day to cats in groups A, D, E, and F, by intermittent subcutaneous injection at a dosage of 60 mg/kg/day divided into three inoculations per day at 8-h intervals to cats in groups B and G, and by the oral route at a dosage of 100 mg/kg/day divided into three treatments given at 8-h intervals to cats in group C. Group E cats also received a single 3.6-mg/kg subcutaneous bolus injection of AZT at 8 h p.i., at the

time that the AZT infusion was begun. The AZT bolus was given to reduce the time necessary to reach the steady-state concentration of AZT in plasma. The amount of AZT in the bolus injection was determined by calculating the amount of drug needed to bring the concentrations in plasma up to the target therapeutic level of approximately 3 μ g/ml on the basis of pharmacokinetic analysis. The significance of efficacy studies was determined by the Fisher exact test. P values of <0.05 were considered statistically significant.

Significant protection from chronic viremia was observed for the cats treated with 60 mg of AZT per kg/day by continuous infusion initiated at -48, 8, and 96 h p.i. (groups A, D, and F; $P = 0.0047$, 0.015, and 0.001, respectively) (Table 1). However, the efficacy of treatment was reduced to 33% (two of six cats) and was not significant ($P = 0.454$) for a separate group (group E) which received a single bolus of AZT (3.6 mg/kg) at 8 h p.i. and then a continuous infusion with 60 mg of AZT per kg/day to parallel the treatment for cats in group D.

When AZT was administered by intermittent subcutaneous injection beginning at -48 h p.i. (60 mg/kg/day; group B), drug efficacy was reduced to 50% (three of six cats) and was not significant ($P = 0.182$) (Table 1). However, when the initiation of intermittent AZT treatment was delayed to 96 h p.i. (group G), the same treatment regimen protected 100% (six of six) of the cats from chronic FeLV viremia ($P = 0.001$). The antiviral efficacy of AZT administered by the oral route (100 mg/kg/day; group C) was not significant, with three of six cats becoming chronically FeLV viremic ($P = 0.182$).

Cats have an age-related susceptibility to FeLV infection (24), in which young cats (8 to 10 weeks of age) are highly susceptible and typically develop serious disease with chronic high-level viremia. We previously have shown that prophylactic AZT therapy prevents chronic FeLV disease in young animals because of a drug-induced vaccine effect (33). AZT therapy appears to attenuate the early stages of FeLV infection, allowing immunity to develop before the virus is widely disseminated.

In the study reported here, substantial changes in drug efficacy resulted from only slight changes in the AZT treatment protocol. When the method of drug delivery was switched from a continuous infusion to intermittent injection, the incidence of chronic antigenemia significantly increased (groups A and B; Table 1). Thus, the delivery of AZT by intermittent injection

during the time of initial exposure to FeLV was significantly less protective than continuous infusion during the same period. This effect was seen only when the intermittent dosing was started close to the time of primary virus infection. When AZT treatment was delayed to 96 h p.i., there was no difference in efficacy between the two treatment regimens (groups F and G; Table 1), both of which gave significant protection.

The differences in drug efficacy may be due to the plasma AZT concentration attained during intermittent drug therapy, which produces a peak and trough pattern, with drug levels being at their highest shortly after drug injection which is followed by a drop at a predictable rate until the next injection. Plasma AZT concentrations were not determined in the present study, but in previous studies of the pharmacokinetics of AZT in cats a 100-mg/kg subcutaneous bolus injection of AZT produced peak concentrations in plasma of between 40 and 140 $\mu\text{g/ml}$ approximately 1 h after injection (32a). By calculation, the predicted peak concentrations in plasma would be between 8 and 28 $\mu\text{g/ml}$ for a 20-mg/kg subcutaneous injection. Drug given at the same dose per day by continuous infusion is maintained at a constant lower concentration in plasma, averaging approximately 2.8 $\mu\text{g/ml}$ (33). Thus, the estimate for the peak concentrations of AZT in plasma following a subcutaneous bolus injection of 20 mg/kg would be between 2.5 and 10 times that for continuous infusion. It is possible that this short-term high AZT concentration in plasma may be toxic to a vulnerable immune cell population being primed by the initial virus infection. We know from in vitro studies that AZT inhibits lymphocyte function (23, 38, 49, 54, 58). The possible loss of response by these FeLV-specific T lymphocytes could affect the animals' ability to prevent the subsequent spread of virus and clear residual FeLV-infected cells.

Delaying AZT treatment until 96 h p.i. appeared to avoid the loss of efficacy, suggesting that at the initial time of virus exposure certain immune elements are particularly sensitive to the drug-related effect. Supporting this conclusion is a separate treatment regimen in which five of six cats were protected from chronic viremia (group D) when a continuous AZT infusion was started at 8 h p.i. However, when a single bolus injection of 3.6 mg of AZT per kg was given at 8 h p.i. along with continuous AZT infusion, protection was reduced to two of six cats (group E) and was not significant.

The results reported here, although statistically significant, are based on small numbers of animals per group. Additional studies will be needed to substantiate the immunomodulatory effects of AZT. In addition, the proposed effects of AZT on the primary immune response, while compatible with those described in other studies (60), are speculative at this time. Alternative explanations relating to the broad toxic effects of AZT on cell proliferation and mitochondrial metabolism should also be considered (51).

The strong correlation between the HIV-specific cell-mediated immune response and long-term survival (4, 5, 8, 10, 11, 15, 40, 42–45) suggests that establishing an effective immune response in HIV-1-infected individuals shortly after virus exposure is perhaps the best hope for resistance to AIDS (26). AZT and other antiviral drug therapies may adversely affect immune function in response to infection (60).

Because the high-dose AZT effect on FeLV susceptibility seems to be associated with a very early stage of infection, the relevance of these observations to individuals with established HIV infections is probably minimal. Health care workers accidentally exposed to HIV are a separate group who may receive postexposure prophylactic AZT therapy shortly after HIV exposure, at a time coincident with the initiation of pri-

mary immune stimulation. Our study suggests that consideration should be given to the timing and dosage of antiviral therapy to this latter group.

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