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Factors influencing zidovudine efficacy when administered at early stages of Friend virus infection in mice

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Summary

Strategies for zidovudine (AZT) administration in retrovirus infection may greatly influence treatment efficacy, especially in the case of early intervention. Antiretroviral activity of AZT in mice infected with Friend leukemia virus (FLV) has been investigated using various experimental protocols. Mice were inoculated with FLV and treated with AZT either 1 or 4 h after inoculation. A dose/effect relationship of AZT therapy was established for two different loads of virus inoculum. The effects of treatment duration (5 or 14 days) and route of administration (b.i.d. subcutaneous injection or administration in drinking water) were also evaluated. In all cases AZT therapy suppressed or reduced virus-induced splenomegaly and increased survival time. AZT therapy was more effective when started 1 h rather than 4 h after virus inoculation. A mutual influence between the dosage of the antiviral drug and the virus inoculum size was observed. A 5-day therapy was inadequate to suppress infection. AZT therapy led to similar results whether administered subcutaneously or in drinking water. The present results suggest that AZT efficacy declines when the inoculum size is increased, when the initiation of treatment is delayed and when treatment duration is shortened.

AZT; Animal model of HIV infection; Friend virus; Chemoprophylaxis

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Introduction

Similarities among retroviruses in man and mouse may help to test different kinds of antiretroviral drugs acting against a common step in the viral replication cycle, particularly in the early phase. We have selected experimental infection of mice with Friend murine leukemia virus (FLV) as a useful tool to evaluate chemoprophylactic intervention. Using this model, antiretroviral efficacy may be evaluated using different parameters. The most important feature is that the degree of splenomegaly is proportional to the virus titer, making FLV infection a quantitative model for evaluation of therapeutic efficacy in vivo. AZT has been shown to be efficient in vitro against retroviruses (Dahlberg et al., 1987; Furman and Barry, 1988) including HIV (Mitsuya et al., 1985) and MuLV (Ostertag et al., 1974), and in vivo during HIV-related disease (Fischl et al., 1987; Dournon et al., 1988). FLV infection of mice was used to study chemoprophylaxis after viral exposure using AZT as a single agent in order to address two questions: does prompt initiation of effective antiviral therapy after viral exposure prevent infection, and what are the best conditions for such therapy? For this purpose, we have determined the influence of timing, dose, treatment duration and route of administration on AZT efficacy after inoculation of mice with FLV. The influence of virus inoculum size, a major factor when considering post-exposure chemoprophylaxis, was also examined.

Materials and Methods

Animals

Male DBA2 mice aged 6 weeks were obtained from Iffa Credo Laboratories (Grenoble, France). Animals were acclimated for one week prior to experimental use. The animals were housed in a protected unit at 21–25°C and supplied with food and water ad libitum.

Virus

The polycythemia-inducing Friend leukemia virus (FLV) was a generous gift from Dr. P. Tambourin, Paris. This FLV complex consists of a mixture of helper Friend murine leukemia virus (F-MuLV) and defective spleen focus-forming virus (SFFV). High titer virus stocks of FLV were prepared from spleen homogenates of mice taken four weeks after infection. Aliquots of cell-free virus suspension were stored at −80°C and titrated for infectivity in vivo by spleen focus assay. Mice were inoculated intravenously with 0.2 ml of virus suspension at two different viral titers.

Zidovudine treatment of mice

Zidovudine (AZT, 3′-azido–3′-deoxythymidine, Retrovir®) was kindly provided by Laboratoires Wellcome, France. Treatment was initiated 1 or 4 h after viral challenge and continued for 5 or 14 days. Two routes of administration were used: subcutaneous injections twice daily at 40 mg/kg/day or per os administration in drinking water at concentrations of 0.1, 0.2, or 0.4 mg/ml.

Evaluation of antiviral efficacy

Each experiment included both treated and untreated mice (placebo groups), either infected or non-infected.

Groups of 10 mice were observed for survival over a 120-day period during which hematocrit and body weight were measured weekly as indicators of drug toxicity.

Groups of 5 mice were sacrificed at day 14 or day 21 post-inoculation. Spleen weight and viral titration in cell-free suspensions of spleen homogenates were used as indicators of virus infection.

The inhibition rate of treatment on the development of splenomegaly was calculated as follows:

$$\text{Inhibition (\%)} = 100 \times \left(1 - \frac{\text{Net spleen weight increase in the presence of drug}}{\text{Net spleen weight increase in the absence of drug}}\right)$$

For measurement of virus titer, the in vivo spleen focus assay was used (Axelrad and Steeves, 1964). Briefly, 0.2 ml of virus suspension, serially diluted (10^{-1} to 10^{-6}), was inoculated by retro-orbital injection. Mice were sacrificed 9 days later. Spleens were weighed and fixed in Bouin's solution. Yellow focal lesions on the planar surface of the spleen were counted macroscopically, and SFFV virus titer (mean number of foci per spleen × dilution factor) were expressed in focus-forming units/ml (FFU/ml).

Statistical analysis

Mean spleen weights of the different groups were compared by variance analysis. Kaplan-Meier survival plots were compared using Mantel-Cox test.

Results

Effect of starting time of AZT administration on therapeutic efficacy

In a first set of experiments, we tested the influence of the time of therapy initiation. Mice were inoculated with 30 FFU of virus and treated either 1 or 4 h

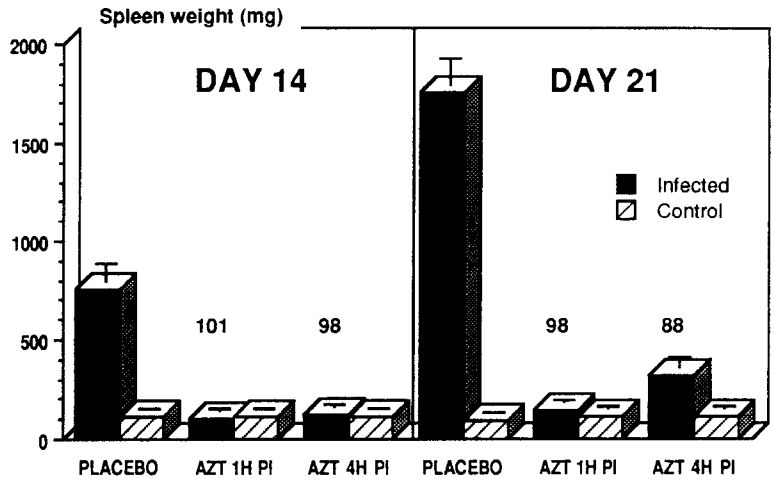

Fig. 1. Effect of initiation time of AZT therapy on inhibition of FLV-induced splenomegaly in DBA2 mice. Groups of 5 mice were inoculated with 30 FFU of virus and AZT therapy (twice-daily subcutaneous injections for 14 days, at 40 mg/kg/day) was started 1 h or 4 h later. Non-infected treated mice were taken as controls. Spleens were taken from mice sacrificed 14 or 21 days after inoculation. Bars are mean spleen weight and standard errors are represented. Values above bars indicate inhibition of splenomegaly (%) attributable to therapy.

later by twice daily subcutaneous injections of AZT at 40 mg/kg/day for 14 days.

Fig. 1 presents mean spleen weights of infected mice and non-infected controls. At day 14, AZT completely inhibited virus-induced splenomegaly whether it was initiated 1 or 4 h after inoculation. In contrast, when the spleens were taken one week after the end of therapy (day 21), such complete inhibition was only observed in the group of mice in which treatment was initiated at 1 h after virus inoculation. In the group treated from 4 h after inoculation, mean inhibition of splenomegaly was significantly lower ($P<0.01$) and reached only 88%. In the same way, mean

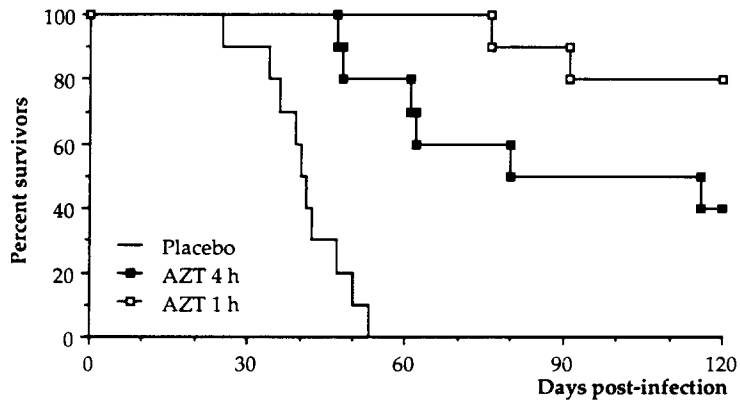

Fig. 2. Effect of initiation time of therapy on survival of FLV-infected mice treated with AZT. Kaplan-Meier plots of virus-infected untreated mice (placebo) and infected mice treated with AZT (40 mg/kg/day) for 14 days starting 1 h or 4 h after inoculation with 30 FFU of virus. Each group contained 10 mice.

survival time and percentage of long-time survivors (Fig. 2) were significantly increased in both groups compared to untreated controls, and a better survival rate was observed for mice treated from 1 h post-inoculation.

Effect of virus inoculum size on therapeutic efficacy of AZT at different dosages

A dose/effect relationship of AZT therapy was established for two different loads of virus inoculum (30 or 300 FFU). Mice were treated from 4 h after inoculation. AZT was given in drinking water at 3 different dosages: 20, 40 or 80 mg/kg/day for 14 days (daily water consumption was used to determine adequate concentrations of AZT in drinking water). AZT appeared to be non toxic at the doses used, as assessed by normal weight gain and absence of hematological toxicity (no change in hematocrit at the end of AZT therapy). As shown in Table 1, when the spleens were observed 14 days after inoculation, the mean spleen weight of infected treated mice was not significantly different from that of non-infected controls, except for mice inoculated with the high virus inoculum and treated with the lowest dose of AZT, where inhibition was only 92%. At day 21, a dose/effect relationship was exhibited and AZT was more effective when the low virus load had been inoculated.

Survival of mice inoculated with a virus load of 30 or 300 FFU is shown in Fig. 3. All the untreated mice died within 60 days. For the mice that had received AZT at 40 mg/kg/day, the percentage of survivors 4 months after infection was greatly reduced in the group inoculated with the high virus load (10% versus 60% in the low virus-load group).

TABLE 1

Effects of oral 14-day AZT therapy at different dosages on splenomegaly induced by FLV in mice

AZT (mg/kg/day)	Non-infected treated mice	Infected treated mice			
		Inoculum size: 30 FFU		Inoculum size: 300 FFU	
	Spleen weight (mg)	Spleen weight (mg)	Inhibition (%)	Spleen weight (mg)	Inhibition (%)
Day 14 post-inoculation					
20	73 ± 5	108 ± 4	96	236 ± 43	92
40	106 ± 10	119 ± 7	98	116 ± 8	100
80	86 ± 9	101 ± 8	98	84 ± 9	100
None	73 ± 4	913 ± 195		2022 ± 146	
Day 21 post-inoculation					
20	105 ± 5	257 ± 96	82	1478 ± 355	30
40	105 ± 8	162 ± 18	93	1171 ± 435	45
80	127 ± 9	122 ± 7	101	135 ± 77	100
None	84 ± 5	1974 ± 222		2291 ± 321	

AZT therapy was initiated 4 h after virus inoculation. Non-infected AZT-treated mice were taken as controls. Values of spleen weights are mean ± standard error for $n = 5$ mice in each group.

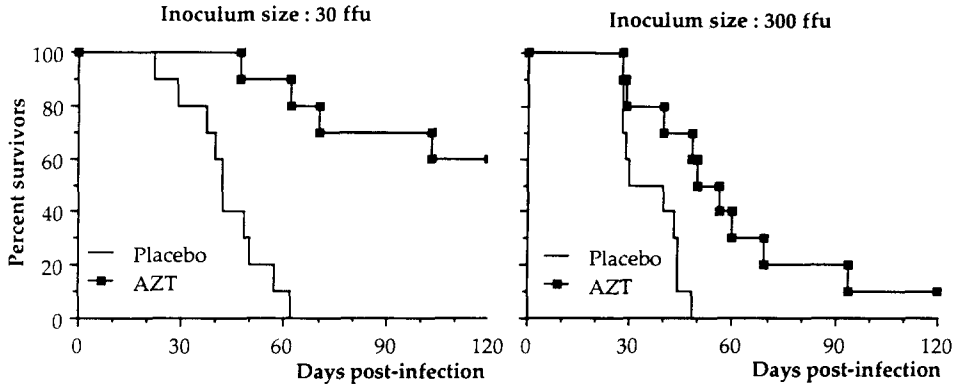

Fig. 3. Effect of virus inoculum size on survival of FLV-infected mice treated with AZT. Kaplan-Meier plots of virus-infected untreated mice (placebo) or infected mice treated with AZT (40 mg/kg/day) in drinking water for 14 days, starting at 4 h post-inoculation. Each group contained 10 mice.

Effect of AZT treatment regimen on therapeutic efficacy

Fig. 4 shows the results of AZT therapy initiated 4 h after inoculation of 30 FFU of virus, and continued for 5 or 14 days. Forty mg/kg/day of AZT was given either in drinking water or by twice-daily subcutaneous injections. Splenomegaly observed at day 21 was only partly inhibited by 5-day therapy, whereas inhibition was almost complete after 14-day therapy. In both cases, the route of administration did not alter the treatment efficacy.

Discussion

Use of prophylactic Zidovudine after accidental exposure to HIV is currently in debate (Gerberding, 1988; Henderson and Gerberding, 1989) and additional studies are needed to help clarify whether AZT can prevent retroviral infection and to optimize strategy of administration. Therefore, post-exposure prophylaxis programs may stand on experience gained from animal studies. Mouse models of retroviral infection provide a screening procedure that allows rapid detection in vivo of useful antiretroviral therapy. Murine leukemia virus (MuLV) infection is a suitable model when using drugs known for their ability to prevent both HIV and MuLV replication in vitro and can provide an in vivo system for testing different therapeutic strategies. In the present work, we have evaluated the potency of AZT against Friend murine leukemia virus in vivo according to different treatment protocols. It has been possible to better define the importance of factors with relevance to chemoprophylaxis after accidental exposure. The time lapse between exposure and treatment seems to be an important factor in therapeutic efficacy; the differences observed when treatment was initiated 1 or 4 h after virus inoculation suggest that therapy has to be initiated in the shortest possible time. We demonstrated a dose/effect relationship for two different virus concentrations of the inoculum.

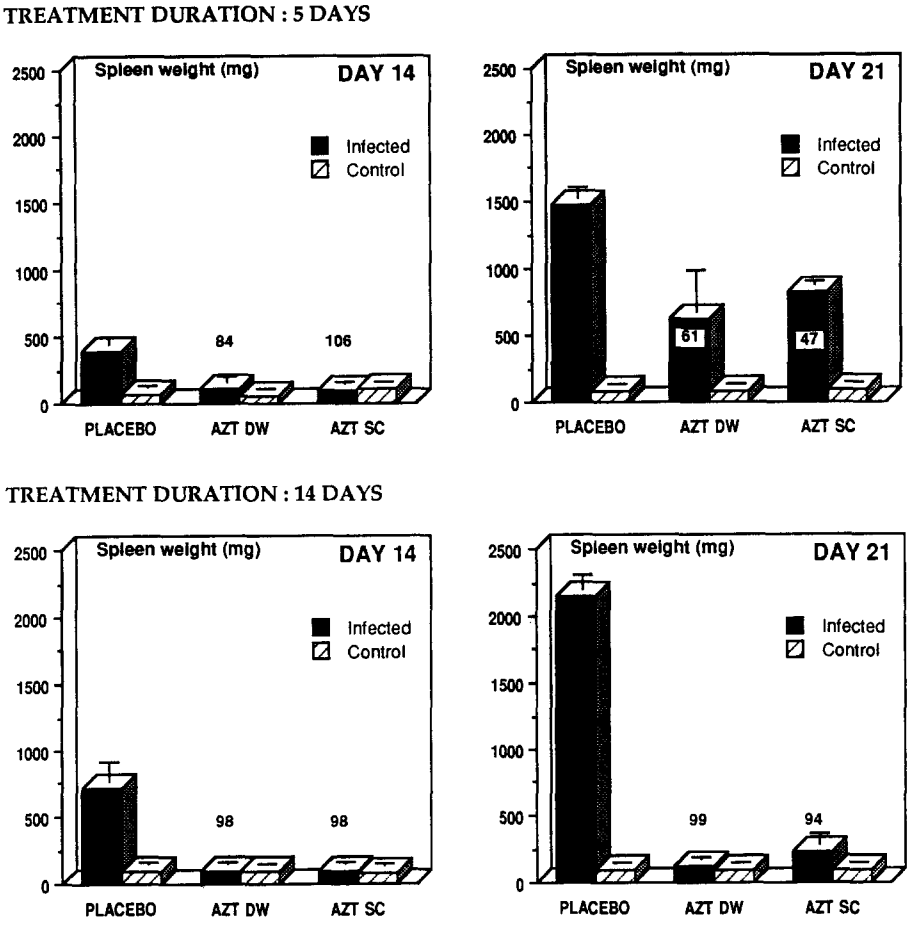

Fig. 4. Effects of AZT treatment duration and mode of administration on reduction of splenomegaly in FLV-infected mice. AZT (40 mg/kg/day) was given from 4 h after inoculation with 30 FFU of virus, either in drinking water (DW) or by subcutaneous injection b.i.d. (SC), for 5 or 14 days. Non-infected treated mice were taken as controls.

Virus load of the inoculum greatly influenced antiviral efficacy, suggesting that adapting the antiviral dosages to the virus inoculum size is important for the optimization of therapy. Five-day therapy was insufficient to suppress disease whereas fourteen-day therapy inhibited almost entirely virus-induced splenomegaly. On the other hand, the two modes of administration used (oral administration in drinking water given ad libitum or b.i.d. subcutaneous injection) led to approximately the same efficacy. Although results from such experiments have to be interpreted with caution, it has been possible to establish relationships between therapeutic regimens and treatment efficacy which may provide guidelines for subsequent clinical trials.

However none of the therapeutic regimens investigated gave complete protec-

tion. Furthermore, in long-term survivors, clearance of the virus inoculum has to be confirmed by sensitive techniques such as polymerase chain reaction to detect integrated proviral DNA. These investigations are currently in progress.

Reviews of results obtained in animal models (Ruprecht et al., 1986; Tavares et al., 1987; Portnoi et al., 1990; Ohnota et al., 1990; Morrey at al., 1990) all demonstrate that AZT reduces or delays occurrence of disease after retroviral infection. However, even when given before infection, as reported by McCune et al. (1990) in the SCID/hu mouse model, AZT fails to completely inhibit virus infection. In the same way, two recent clinical reports (Lange et al., 1990; Looke and Grove, 1990) describe failure of Zidovudine prophylaxis after accidental exposure to HIV. It seems likely that therapeutic efficacy is a consequence of a balance between virus load, drug regimen and the immune defenses of the host. It is possible that the observed failure of AZT therapy is due to inadequate schedules for post-exposure prophylaxis. Another possibility is that AZT is not able to clear the virus inoculum and its efficacy might be greatly enhanced by combination therapy to eliminate remaining infected cells. We have previously shown (Launay et al., 1990) that therapeutic efficacy is substantially enhanced when AZT is administered in combination with the interferon inducer (poly I)·(poly C). Likewise, in a recent report by Ruprecht (1990), it was shown that viremia and disease are prevented after combination therapy of AZT and Interferon α in mice inoculated with Rauscher leukemia virus. In the same direction, new candidate antiretroviral drugs have been recently discovered (De Clercq, 1990). They can provide greater efficacy in vivo and should be evaluated in comparison with AZT.

Acknowledgements

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