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EFFECT OF 3'-AMINO-3'-DEOXYTHYMIDINE ON L1210 AND P388 LEUKEMIAS IN MICE

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The antineoplastic potential of 3'-amino-3'-deoxythymidine (3'-aminothymidine), a compound first synthesized by Horwitz *et al.* (1) and Miller and Fox (2), is under evaluation. Initial studies have indicated that this derivative is cytotoxic, potently inhibiting the replication of L1210 and Sarcoma 180 cells in culture (3). It does, however, lack significant antiviral activity which is in contrast to the selective antiherpes actions of the 5'-amino derivative of thymidine (3,4).

The cytotoxicity of 3'-aminothymidine could be specifically prevented (3) or reversed (5) only by pyrimidine deoxyribonucleosides. In addition, the synthesis of DNA, but not of protein or RNA, was inhibited (5). These findings suggest that 3'-aminothymidine acts as a specific antimetabolite of thymidine. We now report that 3'-aminothymidine effectively inhibits the growth of L1210 leukemia *in vivo* in the absence of significant drug-induced toxicity.

The 3'-aminothymidine used in these studies was prepared as previously described (3). Female BALB/C x DBA/2 (CDF_1) mice, 8- to 12-weeks-old, were obtained from the Charles River Breeding Laboratories, Portage, MI.

Murine L1210 and P388 leukemia cells were passaged weekly in CDF_1 mice. Peritoneal fluid was withdrawn from donor CDF_1 mice bearing 7-day growths and the cell number was determined using a Coulter Counter (Hialeah, FL). In the chemotherapy trials the mice were injected intraperitoneally with a 0.1 ml suspension containing either 10^5 L1210 cells or 10^6 P388 cells. The animals were divided into groups of approximately the same weight and maintained throughout the experiment on Purina Laboratory Chow pellets and water *ad libitum*.

3'-Aminothymidine was dissolved in sterile saline and given by i.p. injection 24 hours after tumor implantation. Subsequent injections were given on the indicated schedules. Control animals were injected on the same schedule with saline only. The mice were weighed during the course of the experiment and the days of death recorded. The median survival time and number of 60-day (long term) survivors were used as measures of drug effectiveness and weight loss was used as an indication of drug toxicity.

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The antileukemic effect of 3'-aminothymidine was both dose and schedule dependent. Table 1 summarizes the effect of the aminonucleoside on L1210 growth in CDF_1 mice.

Table 1. Effect of 3'-Aminothymidine on L1210 Leukemia in CDF_1 Mice

Dose mg/kg	Schedule of Drug Administration	% Increase in Lifespan[a]	Long-Term Survivors[b]	Maximum Weight Loss[c] (%)
20	Daily, days 1-6	0	0/5	2.5
40	Daily, days 1-6	11	0/5	2.0
80	Daily, days 1-6	22	0/5	4.0
160*	Daily, days 1-6	42	1/11	4.5
240	Daily, days 1-6	44	0/6	6.0
320	Daily, days 1-6	72	0/6	4.0
160**	Twice daily, days 1-3	147	1/18	5.7
320***	Twice daily, days 1-3	144	5/12	8.0
640***	Twice daily, days 1-3	128	5/12	8.0
160	Three times on day 1 only	12	0/10	4.0
320	Three times on day 1 only	19	0/10	5.0

[a]Percent increase in lifespan was calculated using the median days of death. In all experiments the median day of death for control animals was 8-9 days.

[b]Animals surviving > 60 days.

[c]The maximum weight loss is expressed as the maximum percent decrease in body weight post drug injection (days 3 or 4).

*Average of two experiments.

**Average of three experiments.

***Average of two experiments (all long term survivors seen in one experiment).

There was a progressive increase in the lifespan of animals treated daily for 6 days as the dose of 3'-aminothymidine was raised from 20 mg/kg to 320 mg/kg. Although there was one 60-day survivor and marked increases in lifespan, based on the estimated number of cells surviving therapy (6), this was not a curative schedule. If the regimen was changed to twice a day for 3 days, the antileukemic effect was dramatically improved. On this schedule increases in the median lifespan of approximately 140% were achieved, and in one experiment

there were a number of long-term survivors. According to the data of Schabel et al. (6), an increase of lifespan of this magnitude suggests that one or fewer L1210 cells survived therapy under these conditions and that cures should be expected. Importantly, this curative schedule was associated with minimal toxicity. A maximum weight loss of 8% was seen.

If the drug was given 3 times a day, but only on day 1, the response was minimal. The maximum increase in lifespan achieved was only 19%.

In a preliminary screen, groups of six CDF_1 mice were injected with P388 leukemia (10^6 cells). Twenty-four hours later they were given 150, 300 and 600 mg/Kg of 3'-aminothymidine twice a day for 3 days. As shown in Table 2, effective but not curative therapy was achieved.

Table 2. Effect of 3'-Aminothymidine on P388 Leukemia in CDF_1 Mice

Dose mg/kg	Schedule of Drug Administration	% Increase in lifespan[a]	Long-Term Survivors[b]
none	-	-	0/6
150	Twice daily, days 1-3	22	0/6
300	Twice daily, days 1-3	22	0/6
600	Twice daily, days 1-3	33	0/6

[a]Percent increase in lifespan was calculated using the median days of death which for control animals was 9 days.
[b]Animals surviving > 60 days.

The data presented here show that 3'-aminothymidine is a very effective antileukemic drug in vivo. Most importantly, this therapeutic effect is achieved with very little drug-induced toxicity. The maximum weight loss was only 8%. It is not surprising that more frequent administration of 3'-aminothymidine produced a greater antitumor effect. Nucleosides are rapidly metabolized and excreted and adequate blood levels are difficult to maintain. In addition, if 3'-aminothymidine is phase-specific, then appropriate scheduling would be critical (7).

3'-Aminothymidine may be less effective against P388 than L1210 leukemia. However, a 10-fold greater burden of P388 cells was used and no attempts to optimize the regimen were made.

Preliminary studies reported by Fischer et al. (5) indicated that 3'-aminothymidine is a potent inhibitor of DNA synthesis in L1210 cells in culture. Inhibition of DNA biosynthesis measured by the uptake of [methyl-^{3}H]-thymidine and [2-^{3}H]-adenine was marked at concentrations which inhibited cell growth by 65%. Neither RNA nor protein synthesis was altered detectably at a 25-fold greater drug concentration. 3'-Deoxy-3'-fluorothymidine is similar to 3'-aminothymidine in that it has a fluorine atom in place of the 3'-amino function and Langen and co-workers have shown that the

fluoro derivative of thymidine is also a selective inhibitor of DNA synthesis (8). The fluoro derivative is metabolized to the 5'-triphosphate and subsequently incorporated into DNA, where it is likely to terminate chain growth (9). Whether 3'-aminothymidine acts similarly is under investigation. Thus, 3'-aminothymidine is an effective anticancer agent in vivo and is a promising candidate for further evaluation.

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