Inhibitory Effect of Adenosine Dialdehyde on in Situ Murine Neuroblastoma Growth

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ABSTRACT

The effect of adenosine dialdehyde (AD) on in situ tumor growth and host survival was evaluated in the C1300 murine neuroblastoma tumor model prepared by implantation of murine neuroblastoma cells into A/J mice. AD was administered s.c. by one of the following treatment regimens: regimen A, single daily dose for 5 days; regimen B, minipump infusion for 7 days; regimen C, minipump infusion for 14 days; regimen D, minipump infusion for two 7-day periods interspersed by a 7-day drug free interval. AD doses of 1.5 to 2.5 mg/kg/day infused over a 7-day period (regimen B) significantly increased the mean life span of tumor bearing mice from 20.9 ± 1.2 days (mean ± 2 SEM) in diluent treated controls to 35.3 ± 2.1 days in AD treated animals (mean increase ± 2 SEM: 69 ± 10%; P < 0.0001). This treatment regimen also produced a 56 ± 13% decrease in tumor diameter (P < 0.0001). Administration of AD for two 7-day infusion periods, interspersed by a 7-day drug free interval (regimen D), increased mean life span 80% (controls, 21.3 ± 4.4 days; AD treated 38.4 ± 5.6 days; P < 0.0005). Hematopoietic toxicity was not observed when doses between 2 and 3 mg/kg/day of AD were infused for 7 days (regimen B). These data suggest that steady state infusions of AD can significantly suppress murine neuroblastoma tumor growth with little systemic toxicity. In contrast, single daily injections of AD were ineffective and toxic to the tumor bearing host.

INTRODUCTION

The MNB1 model reflects its human counterpart in morphology, catecholamine biosynthesis and metabolism, metastatic spread, host lethality, and response to chemotherapy (1, 2). This model has been used to evaluate the effects of host age, tumor load, sympathectomy, and purine nucleoside analogues on the growth and differentiation of MNB (3, 4).

The purine nucleoside analogue AD has been shown to suppress MNB cell replication in tissue culture with concentrations of 1.5 µM producing 50% inhibition (3). Substitution of adenine with other bases such as inosine, uridine, cytidine, and guanosine or reduction of AD yielded compounds with 1000-fold less cytotoxic activity. Analogues of AD modified in position 5', so that phosphorylation at this site was precluded, were also cytotoxic and, in common with AD, they inhibited S-adenosylhomocysteine hydrolase in intact MNB cells (3, 5).

This investigation has demonstrated that AD exerts a potent inhibitory effect on the in situ growth of established MNB tumors, prolongs the life span of tumor bearing mice, and does not suppress hematopoiesis when administered by steady state infusion.
in life span were computed for each experiment by comparing mean values for the drug treated animals with pooled data from the diluent treated animals. Student's \textit{t} test was utilized to determine the significance of each comparison.

A formal 50% lethal dose was not determined, since all animals were tumor bearing, and it was therefore impossible to determine whether death was due to the tumor or exclusively to the effects of AD. Drug induced toxicity was estimated by calculating the dose of AD which decreased the life span of 50% of drug treated tumor bearing mice to 24 days or less after implantation of MNB cells. This life span was selected because 24 days represented the life span (mean ± 2 SEM) of control (diluent treated) tumor bearing mice. This dose was calculated by linear regression analysis of the log [AD dose] against survival (survival encoded as follows: 0 = survival greater than 24 days; 1 = survival less than 24 days).

RESULTS

Toxicity of AD in Murine Neuroblastoma Bearing Mice. The daily s.c. dose of AD given for 5 consecutive days (regimen A) that decreased the life span of 50% of drug treated tumor bearing mice to 24 days or less was 38 µg/mg/day with 95% confidence intervals of 18 to 81 (Table 1). This regimen produced a negligible effect on tumor diameter and animal life span. When AD was infused for a 7-day period via minipump (regimen B), the estimated dose required to decrease life span of 50% of drug treated tumor bearing mice to 24 days or less was 3.6 µg/mg/day with 95% confidence intervals of 1.2 to 10.5 (Table 1).

Effect of Adenosine Dialdehyde on Murine Neuroblastoma Growth in Situ. A variety of treatment regimens utilizing AD were investigated to determine the optimal infusion schedule for inhibiting MNB tumor growth. The response of MNB tumors to a 7-day infusion of AD (regimen B) at doses ranging from 1 to 4 mg/kg/day are presented in Fig. 1. Tumor growth was arrested and average tumor diameter remained static at a dosage of 1 mg/kg/day. A dose dependent reduction in tumor size was observed following infusion of doses greater than 1 mg/kg/day. The increase in mean life span above control animals was 28% (\(P = 0.03\)), 57% (\(P < 0.0005\)), 26% (\(P = 0.10\)), and 45% (\(P = 0.01\)) for doses of 1, 2, 3, and 4 mg/kg/day, respectively. The effect of longer infusion periods on tumor response to AD was evaluated by use of regimen C (14-day infusion) and D (infusion for two 7-day periods interspersed by a 7-day drug free interval). The suppression of MNB tumor growth was not altered by increasing the duration of infusion from 7 to 14 days (Fig. 2). Despite a 2-fold increment in total AD dosage, there was no corresponding augmentation of tumor regression. Results similar to regimen B were obtained with regimen C, which increased mean life span above control animals to 30% (\(P = 0.04\)), 20% (\(P = 0.03\)), and 33% (\(P < 0.0005\)) for doses of 1, 2, and 3 mg/kg/day, respectively. A sustained arrest of tumor growth was produced by regimen D when a dosage of 2 mg/kg/day was administered (Fig. 3). In addition,
the mean life span of the treated mice was increased 80% above that of controls (P < 0.0005).

The influence of early treatment with AD on subsequent tumor growth and host survival was also evaluated (Fig. 4). In this experiment, 7-day infusions of AD (2 mg/kg/day) were started at the time of tumor implantation (day 0) or on post-implantation day 5 or 9. Tumor growth was suppressed in each group although no cures were observed, even when AD administration was begun on day 0. AD infusions initiated on day 0 extended the average life span from 21.7 days to 33.4 days, a 54% increase above untreated controls (P < 0.0005). Animals in whom treatment was initiated on day 5 had a 100% increase in mean life span (P < 0.0005), whereas those started on day 9 had a 60% increment above controls (P < 0.0005).

Determination of “Optimal Therapeutic Dose” of AD. The “optimal therapeutic dose” of AD was determined by comparing the changes in life span, tumor diameter, and body weight produced by minipump infusions of AD at doses between 0.5 and 4.5 mg/kg/day (Fig. 5; Table 2). Doses of AD between 1.5 and 2.5 mg/kg/day increased the mean life span 69% above control animals, caused a 17% loss of body weight, and reduced tumor diameter 41% from values at the onset of AD therapy. Doses exceeding 1.5 to 2.5 mg/kg/day produced greater toxicity, as evidenced by an increased loss of body weight and a smaller increment in mean life span, without a significant augmentation in tumor diameter reduction.

Hematological Toxicity. The effect of 7-day infusions of AD (regimen B) at doses of 1 or 2 mg/kg/day on the WBC and hemoglobin concentration of the mice was minimal. A slight but significant decrease in the WBC was observed after 7 days in animals receiving AD at a dose of 3 mg/kg/day (Table 3). An equivalent decrease in the hemoglobin concentration of control and AD treated mice was observed. This was presumably due to blood sampling and not to the AD treatment since the hemoglobin concentrations in these two groups at 7 days were not significantly different.

DISCUSSION

The dialdehydes of purine nucleosides have been shown to suppress the in situ growth of murine tumors such as Ehrlich ascites, Sarcoma 180, L1210 leukemia, and adenosarcoma 755 (8, 9). AD inhibited the replication of L1210 leukemia cells and increased life span approximately 40% when administered i.p. at a dose of 20 mg/kg/day until death (8). Inosine dialdehyde (diglycoaldaldehyde) has been clinically evaluated in phase I trials, with limited efficacy (10).

The mechanisms and enzymatic sites of action of purine nucleoside dialdehydes have been actively investigated in recent years. This laboratory has previously demonstrated AD to be a potent inhibitor of MNB in tissue culture (50% inhibitory concentration, 1.5 μM) (3). Other nucleoside dialdehydes including those prepared from inosine, cytidine, uridine, and guanosine exhibited much less activity. Borohydride reduction of AD also rendered the compound inactive. Recently, AD was shown to be a potent inhibitor of S-adenosylhomocysteine hydrolase in MNB and mouse L929 cells following in vitro incubation (5, 11). Adenosine dialdehyde also exerts an indirect, and possibly less significant inhibitory effect on MNB protein carboxymethyltransferase activity (12). Adenosine dialdehyde and its mono- and triphosphate derivatives can inhibit mamalian or yeast ribonucleotide reductase and hence DNA synthesis at mM concentrations in vitro; the 50% inhibitory con-
centration of these compounds are approximately 1.1 and 0.1 mm, respectively (13, 14). However, ribonucleotide reductase activity was not decreased following incubation of intact MNB cells with AD at a concentration of 1 μM (5).

 Analogues of AD which lack the 5'-hydroxyl group and cannot undergo phosphorylation are almost equipotent inhibitors of MNB growth and S-adenosylhomocysteine hydrolase in vitro. This observation has suggested that phosphorylation is not a prerequisite for cytotoxic activity (3, 5).

 The inhibitory effects of AD on MNB cell replication and enzyme activity appear to be modulated by its uptake into the cell. An energy dependent nucleoside transport system may be operational in facilitating the movement of AD across the MNB cell membrane, since the uptake of [3H]AD is temperature dependent and is partially inhibited by the nucleoside transport inhibitor nitrobenzylthioinosine (15).

 A large number of potential and established antineoplastic compounds have been evaluated using the in situ MNB model system (1, 16–23). With the exception of vincristine, which is active in human neuroblastoma, the response of MNB in situ to chemotherapeutic agents appears to parallel that of human neuroblastoma. The increase in life span and inhibition of tumor growth produced by AD following continuous infusions was comparable to that seen with other agents currently being used for the treatment of human neuroblastoma. The inactivity of AD when given as daily injections may result from its rapid deamination in peripheral tissues to the less active metabolite, inosine dialdehyde. Rapid metabolism would effectively reduce the half-life of AD, so that in comparison to single daily injections, a continuous infusion of AD would be expected to increase the concentration-time product and enhance cytotoxicity.

 The consistent reduction of large established MNB tumors and the prolongation in life span of tumor bearing animals produced by infusions of AD suggest that this compound may merit further evaluation against human neuroblastoma tumors xenografted in nude mice.

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