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Suppression of Growth of Mouse Neuroblastoma and A-10 Adenocarcinoma in Newborn Mice Treated with the Ganglionic Blocking Agent Chlorisondamine*

EWA CHELMICKA-SCHORR† and BARRY G. W. ARNASON

Department of Neurology, University of Chicago and the Pritzker School of Medicine, Chicago, Illinois 60637, *U.S.A.*

Abstract—*Chlorisondamine, an agent which blocks postnatal maturation of the sympathetic nervous system, markedly suppressed growth of C-1300 neuroblastoma and A-10 breast adenocarcinoma in newborn mice.*

Tumor growth in adult mice was not affected by treatment with chlorisondamine.

INTRODUCTION

Biochemical and morphologic maturation of sympathetic neurons occurs postnatally in mice and rats. An intact preganglionic innervation is essential for normal postnatal sympathetic neuronal development. Deafferentation by surgical transection of preganglionic nerve trunks prevents the developmental increase in tyrosine hydroxylase (T-OH), dopa decarboxylase (DDC), and dopamine-β-hydroxylase (DβH) normally observed in sympathetic neurons in the postnatal period [1–3]. A similar effect can be achieved by chemical means. Chlorisondamine (Ecolid), a nicotinic ganglionic blocking agent, prevents afferent input into adrenergic neurons by competing with acetylcholine for receptor sites. Treatment of newborn mice with chlorisondamine blocks the expected postnatal rise in T-OH, DDC, and DβH in sympathetic neurons and causes a net reduction in neuron number and ganglion volume [4–6]. Chlorisondamine does not alter enzyme activity in the sympathetic ganglia of adult animals nor does it inhibit T-OH activity *in vitro* [4].

Recently we reported that sympathetic axotomy produced by pretreatment of adult mice with 6-hydroxy-dopamine (6-OH-DA) significantly suppresses growth of C-1300 neuroblastoma (NB) but does not affect growth of the A-10 breast adenocarcinoma (A-10) [7]. Total permanent sympathectomy produced by pretreatment of newborn mice with 6-OH DA also suppresses NB growth significantly and suppresses growth of A-10 to a lesser extent. NB growth is significantly augmented in mice pretreated with nerve growth factor as newborns [8]. NGF treatment in the newborn period causes hypertrophy of the sympathetic nervous systems (SNS) and increased synthesis of adrenergic neurotransmitters in the sympathetic ganglia [9]. The experiments outlined above suggest a modulatory role for the SNS in tumor growth. We now report the effect of treatment with the ganglionic blocking agent chlorisondamine on NB and A-10 growth in mice.

MATERIALS AND METHODS

Six week old male and pregnant female A/J and A/HeJ mice were obtained from Jackson Laboratory, Bar Harbor, Maine. NB was obtained from Jackson Laboratory and the A-10 tumor from Dr. K. McCully of the Department of Pathology at Massachusetts General Hospital. Both tumors were passaged by serial S.C. transfer. Chlorisondamine was a gift from CIBA-GEIGY Corp., Summit, New Jersey.

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†To whom requests for reprints should be addressed, at Department of Neurology, University of Chicago, Pritzker School of Medicine, Division of the Biological Sciences, 950 East 59th Street, Chicago, Illinois 60637.

New born or adult mice were treated with Ecolid in a dose of 5 μg/g of body weight injected s.c. twice a day for two weeks. Treatment of newborns was begun on the second day of life. Controls received twice daily s.c. saline. Tumor cell suspensions of NB and A-10 were prepared by gentle homogenization of s.c. tumors. Suspension of NB or A-10 tumor cells containing 5×10^5 viable cells/ml of BSS solution were prepared and 0.02 ml (10^4 cells) was injected in the flank on the 4th day after treatment with Ecolid was started. Ten days later tumors were removed and weighed. Statistical significance of the difference in mean tumor size between treated and control groups was calculated using Students *t*-test.

RESULTS

Ecolid treatment of tumor-bearing newborn mice significantly slowed growth of neuroblastoma and A-10 breast adenocarcinoma. It did not influence growth of either tumor in adult animals (Fig. 1).

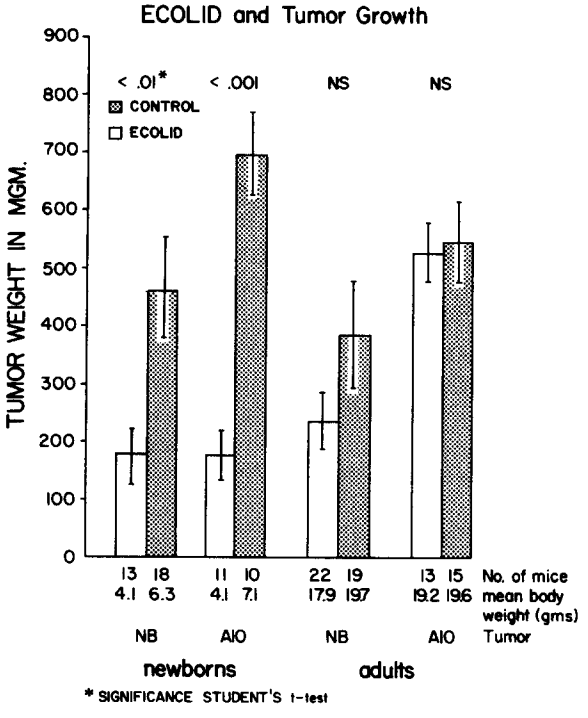

Fig. 1. Tumor growth in Ecolid treated mice. Vertical bars give the standard error of the mean. Significance was calculated using Student's t*-test.*

Ecolid treated newborn A/J and A/HeJ mice and adult A/J mice were smaller than controls. When tumor weights in individual newborn mice were calculated as a percentage of body weight mean values were 3.7% for NB in Ecolid treated mice versus 6.9% for controls and 3.8% for A-10 tumor in Ecolid treated mice versus 9.9% for controls.

DISCUSSION

The data presented show that NB and A-10 tumor growth is suppressed in mice in which biochemical and morphologic maturation of the SNS is prevented by treatment with the ganglionic blocking agent chlorisondamine in the newborn period but treatment with this agent fails to effect tumor growth in adult mice. The mature sympathetic nervous system is relatively refractory to deafferentation by chlorisondamine. Since the results differ in newborn and mature animals a direct influence of chlorisondamine on tumor cells is unlikely and suggest rather that the suppression of tumor growth observed in immature mice relates directly to impaired SNS function.

Chlorisondamine treated mice were smaller than littermate controls, an observation which we and others have made earlier in sympathectomized mice. An intact SNS may be necessary for normal growth. Treatment of fore-limb amputated newts with guanethidine, an anti-adrenergic agent, or with atropine, an anti-cholinergic agent, significantly retards limb regeneration [10]. Proliferation and differentiation of blastemal cells was markedly affected by the treatments described above.

Whether normal growth and tumor growth both of which are retarded in sympathectomized animals are related phenomena is not known. We know of no studies other than our own in which SNS function has been linked to tumor growth. In one study NB growth in the leg muscles of mice with a cut sciatic nerve (a mixed motor and sensory nerve) was reported to be slowed in 20% of instances [11].

It has been suggested on the basis of limited evidence that vasoactive amines may favor new vessel formation [12]. Possibly in the absence of sympathetic transmitter release the neovascularization essential for tumor growth is retarded.

It must be conceded that we have no explanation for our findings at present. At the same time the results reported in this communication are consistent with those obtained in our previous experiments and provide additional evidence for a regulatory role for the SNS in tumor growth.

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