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Letter to the Editor

Treatment of Mouse Neuroblastoma with Methyldopa*

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C-1300 mouse neuroblastoma (NB) shares many morphological and biochemical properties with immature neurons. NB cells synthesize, matabolize and store neurotransmitters and contain enzymes involved in neurotransmitter synthesis and degradation. NB tumor cells also possess transmitter receptors and high affinity uptake systems for neurotransmitters and their precursors [1, 2]. Murine NB resembles human NB in many respects and it is commonly employed as an experimental model for the latter.

Earlier, we showed that the sympathetic nervous system (SNS) influences growth of NB *in vivo*. Sympathetic axotomy in adult mice and total chemical sympathectomy in newborns produced by pretreatment with 6-OH dopamine (6-OH DA) slowed NB growth significantly in treated mice [3]. The treatment effect was specific for NB; growth of mouse breast adenocarcinoma (A10) or mouse melanoma was unchanged. In contrast, pretreatment of newborns with nerve growth factor (NGF) augmented NB growth substantially [4]. NGF produces overgrowth of sympathetic ganglia and hyperinnervation of the periphery. Treatment of newborns with chlorisondamine, an agent which blocks afferent cholinergic input into sypathetic ganglia and thus arrests maturation of the SNS, also slowed NB growth [5]. The experiments outlined above suggest a modulatory role for the SNS in NB growth. We now report that treatment with the adrenergic blocking agent, methyldopa [levo-3-4(3, 4 dehydroxyphenyl)-2-methylalanine, ethyl ester hydrochloride] (Aldomet), a drug employed for treatment of hypertension, slows the growth of s.c. implanted NB. Wick has independently shown that i.p. injection of the methyldopa analogue L-dopa methyl ester, prolongs survival of mice bearing i.p. NB [6] and that injection of methyldopa prolongs survival of mice bearing L1210 or P388 leukemias [7].

Tumor cell suspensions of NB and of A10 were prepared by homogenization of s.c. tumors. A suspension containing 10^5 cells/ml was injected s.c. into the flank. NB tumor was injected into A/J and A10 tumor into A/HeJ mice. Experimental animals received Aldomet i.p. (Merck, Sharp and Dohme) twice daily beginning on the day of tumor injection and continuing throughout the experiment. Controls received 0.9% NaCl solution. After 14 days, the tumors were excised and weighed. Significance of the difference in mean tumor weight between treated and control groups was calculated using Student's *t*-test.

For *in vitro* studies, NB clone N2A cells and A10 cells were cultured in Dulbecco's modified MEM with L-glutamine and 20% fetal bovine serum. Cells were plated in 0.3 ml flat bottom microtiter wells (10,000 cells per well) and maintained at 37°C in a 5% CO_2, 95% room air humidified incubator. Twenty-four hr later, medium was removed and serum-free medium containing the methyldopa analogue L-dopa methyl ester (Sigma Chemical Company) in three different concentrations and tritiated thymidine 1 μCi/ml (sp. act. 6.7 Ci/mmole) was added. After 60 min incubation, cultures were harvested with a Mash II apparatus (Microbiological Associates) and

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Table 1. *Effect of* L-*dopa methyl ester on thymidine incorporation by tumor cells* in vitro

Concentration of α-MD (nM)	NB tumor Counts/min ± S.E.M.*	NB tumor Decrease in incorporation of ^{3}H thymidine (%)	A10 tumor Counts/min ± S.E.M.*	A10 tumor Decrease in incorporation ^{3}H thymidine (%)
0	8153 ± 625 (10)	—	5351 ± 436 (10)	—
3.0	361 ± 33 (5)	95.6	328 ± 28 (5)	93.9
1.5	706 ± 142 (5)	91.4	492 ± 33 (5)	90.9
0.5	2141 ± 184 (5)	73.8	805 ± 62 (5)	85.0

*Number of cultures studied is given in parenthesis.

dry filters placed in scintillation fluid containing toluene and Omnifluor (New England Nuclear). Radioactivity was measured in a liquid scintillation counter. Values were expressed as counts/min and the percentage of inhibition of thymidine incorporation compared to controls was calculated.

Twenty-five mg of Aldomet killed all six mice tested within a few minutes of injection. A dose of 2.5 mg twice daily in a pilot experiment (three treated and three control mice) reduced tumor size from 260 mg to 140 mg. This difference was not significant. Aldomet treatment at a dose of 7.5 mg twice daily significantly slowed growth of NB, but growth of A10 tumor was not influenced (Fig. 1). The vertical bars give the S.E. of the mean. Aldomet-treated mice were slightly smaller than littermates treated with saline and some had mild diarrhea; no other untoward effects were noted. *In vitro* L-dopa methyl ester is equally toxic to NB and A10 (Table 1).

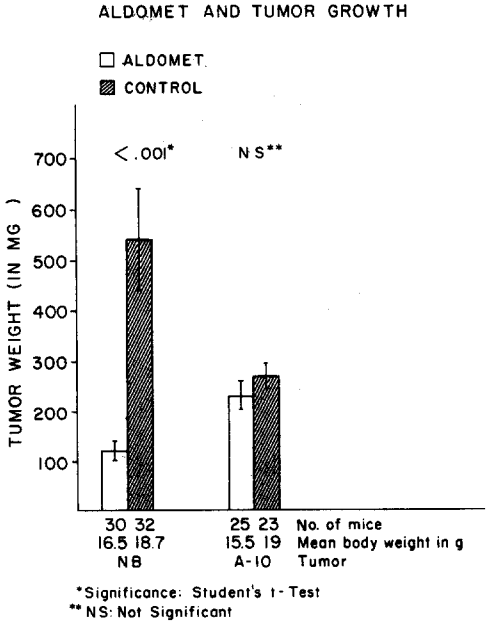

Fig. 1. *Aldomet and tumour growth.*

Methyldopa differs structurally from the aminoacid L-dopa by addition of a methyl group on the α-carbon. *In vivo* methyldopa is taken up by adrenergic nerves, is metabolized like natural dopa, and false neurotransmitters such as methyldopamine and methylnorepinephrine are produced. Treatment with methyldopa leads to marked depression of norepinephrine in adrenergic nerves in both the central and peripheral nervous systems [8–14]. The mechanism of norepinephrine depletion is not understood.

Depletion of norepinephrine in adrenergic endings creates a model similar to the chemical axotomy which we showed previously suppresses growth of NB [3]. However, tumor growth is slowed to an even greater extent in Aldomet treated than in axotomized mice. This suggests that Aldomet affects the tumor directly. Since C-1300 NB cells produce dopamine and norepinephrine, it is highly possible that methyldopa is taken up by the tumor and changes tumor metabolism by depleting neurotransmitters and/or producing false neurotransmitters. Abnormal tumor metabolism could in turn influence tumor growth.

Our *in vitro* experiments on the effect of L-dopa methyl ester on a C-1300 NB cell line agree with those of Wick [6]. L-Dopa methyl ester is toxic to NB cells in culture. L-Dopa methyl ester is as toxic to A10 cells as to NB cells in culture, yet growth of A10 tumor *in vivo* is unaffected by Aldomet.

We offer no definitive explanation for our findings, especially since the mechanism of action of methyldopa is not fully understood. The present data do extend our earlier evidence that sympathetic blocking agents suppress NB growth. These findings may ultimately have implications for therapy of human NB.

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