

Toxicity of Methyldopa (Aldomet) to Mouse Neuroblastoma Cells *in Vivo*¹

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ABSTRACT

The adrenergic blocking agent methyldopa (Aldomet) is toxic to C-1300 neuroblastoma cells *in vivo*.

Four injections of Aldomet at a dose of 7.5 mg/injection were given over a period of 24 hr to C-1300 neuroblastoma-bearing mice. This treatment killed a significant proportion of the C-1300 neuroblastoma cells. Flow cytometric data suggest that sensitivity of tumor cells to Aldomet is not related to the cell cycle.

INTRODUCTION

NB³ is the most common solid tumor of infancy. There is no effective treatment for this tumor at present. NB and the SNS have the same embryological origin, the neural crest. NB shares several neural properties with sympathetic neurons, including the catecholaminergic metabolic pathway. We reported previously that the SNS modulates growth of NB. Chemical sympathectomy and axotomy suppress growth of experimental mouse C-1300 NB significantly and specifically. To the contrary, pre-treatment of newborn mice with nerve growth factor, which causes hypertrophy of the SNS, augments NB growth significantly (1, 4). Treatment of the newborn mouse with chlorisondamine, an agent which blocks afferent cholinergic input into sympathetic ganglia and arrests maturation of the SNS, also slows C-1300 NB growth (2). Daily treatment with the adrenergic blocking agent methyldopa (Aldomet), a drug commonly used as an antihypertensive agent, significantly slows growth of s.c. implanted C-1300 NB (3). Treatment is at least relatively specific for this neural tumor, since Aldomet does not influence growth of A-10 adenocarcinoma *in vivo* (3). Treatment with the methyldopa analogue L-dopa methyl ester has been shown previously to prolong survival of mice bearing i.p. C-1300 NB (11). We now present data concerning the effect on tumor cells achieved by a 1-day treatment with Aldomet of mice bearing solid C-1300 NB tumors. Data are illustrated by flow cytometry.

MATERIALS AND METHODS

Six experiments were done. In each experiment, 4 to 6 adult male A/J mice were given s.c. injections in the flank with 10⁵ living C-1300 NB cells suspended in 0.1 ml of HBSS without Ca²⁺ or Mg²⁺. Tumors were permitted to grow for 7 days, at which time the mice were divided into treatment and control groups. Treated mice were given 4 i.p. injections of Aldomet for i.v. injection (Merck Sharp and Dohme, West Point, Pa.) over a 24-hr period. Each injection contained 7.5 mg of Aldomet in 0.15 ml of vehicle. Control mice received 4 i.p. injections of 0.15 M NaCl. Tumors were removed within 3 hr of the last Aldomet injection and

weighed individually, and then tumors from treated mice were pooled, as were tumors from controls. Cells from the tumors were dispersed by gentle teasing, passed through a 74- μ m stainless steel mesh screen, suspended in HBSS, and divided into aliquots. Living and dead tumor cells (readily distinguished from host lymphocytes and macrophages by their larger size and more abundant cytoplasm) were counted in one aliquot following exposure to 0.08% trypan blue in HBSS.

Additional aliquots were analyzed by flow cytometry. In the first 3 experiments, one aliquot was treated with DNase (0.1 mg/ml) and trypsin (0.25%) to lyse dead cells prior to flow cytometric analysis. DNase and trypsin-treated aliquots were spun down, and the cell pellets were resuspended in 0.2 ml of HBSS solution containing 10% fetal bovine serum. Untreated cells were also in HBSS containing 10% fetal bovine serum. To these samples was added 0.5 ml of a solution containing 0.1% (v/v) Triton X-100, 0.1 N HCl, and 0.15 M NaCl. Cells were stained 45 sec later by adding 1.5 ml of a solution containing 0.15 M NaCl, 1 mM sodium EDTA, and AO (10 μ g/ml) in 0.2 M phosphate-0.1 M citrate buffer (pH 6.0). The fluorescence intensity of individual cells was measured using a FC-200/4800A flow cytometer (Ortho Diagnostic Systems, Inc., Westwood, Mass.). Fluorescence signals were generated by the cells as they passed the 488-nm beam of an argon ion laser. Green fluorescence from DNA-bound AO was collected in a band from 515 to 575 nm, while red fluorescence from RNA-bound AO was collected in a band from 600 to 650 nm. The individual DNA and RNA content of 5000 tumor cells was recorded on a computer disc for cell cycle analysis. Each cell was assigned a position in the cell cycle. Discrimination between living and dead cells and cells in the G₁, S, and G₂ and M stages of the cell cycle was done using a computer interactive program (for details of the technique, see Refs. 6 and 10). The position of dead cells was determined by comparison of aliquots treated with DNase and trypsin with aliquots not so treated. Once the distinct position of dead cells had been learned, the DNase and trypsin treatment step was omitted in the last 3 experiments.

C-1300 NB is an aneuploid tumor. Host diploid cells sequestered in positions distinct from those of the tumor cells because of the lower DNA content of host cells. The flow cytometry display was set up so as to exclude host cells from the picture. Host cells were not lysed to any appreciable extent by DNase and trypsin treatment.

The significance of the differences between the proportions of living and dead cells in the treatment and control groups was calculated using the paired *t* test.

RESULTS

Tumors from 16 Aldomet-treated mice weighed 129 \pm 36 (S.E.) mg. Tumors from 14 control mice weighed 166 \pm 32 mg. The difference between groups is not significant.

For tumors from Aldomet-treated mice, the proportion of dead tumor cells (determined by the trypan blue exclusion test) was 71.0 \pm 2.9% (6 experiments); for tumors from control mice, it was 46.9 \pm 1.8%. This difference is highly significant (*p* < 0.001).

Flow cytometric analysis showed 68.4 \pm 5.3% dead cells in tumor cell suspensions from Aldomet-treated mice (same 6 experiments); 50.0 \pm 5.5% dead cells were found in tumor cell suspensions from controls. This difference is again significant (*p* < 0.02). Data from a representative experiment is illustrated in Chart 1. There was no meaningful difference in the percentage

¹ Supported by NIH-NINCDS-18413.

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³ The abbreviations used are: NB, neuroblastoma; SNS, sympathetic nervous system; HBSS, Hanks' balanced salt solution; AO, acridine orange.

Received May 14, 1982; accepted April 22, 1983.

of dead cells when counts obtained by the 2 methods were compared.

Using a computer interactive program, the proportion of cells in the G₁, S, and G₂ and M stages of the cell cycle was determined

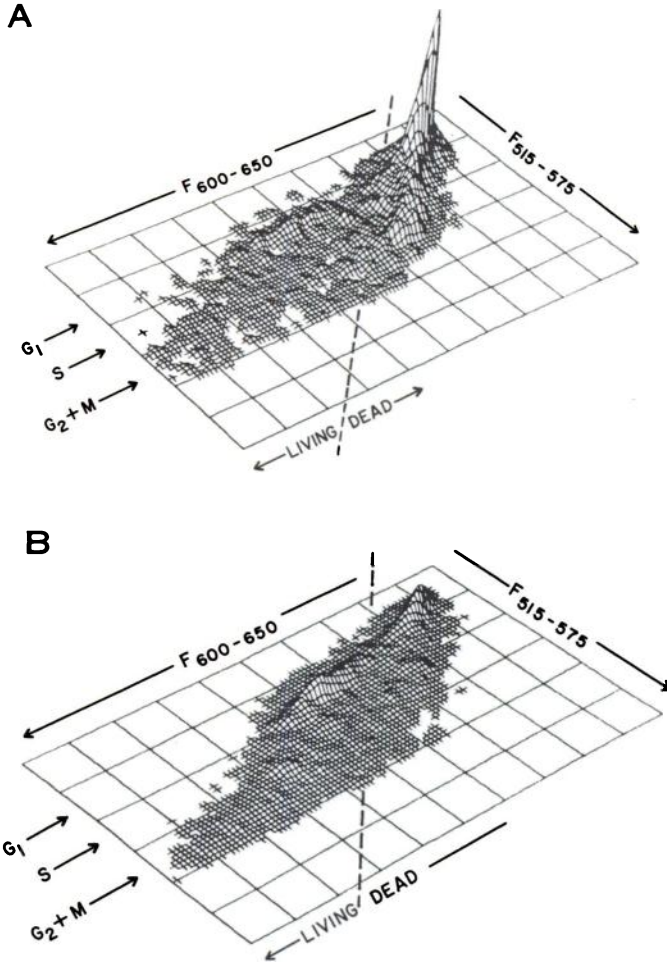


Chart 1. Representative 2-parameter histogram of the relative DNA ($F_{515-575}$) and RNA ($F_{600-650}$) content of 5000 C-1300 NB cells from solid tumors. A, C-1300 NB cells from mice treated with Aldomet; B, C-1300 NB cells from control mice treated with 0.15 M NaCl. Height of the curve above any point in the DNA-RNA plane indicates the relative number of cells containing that amount of DNA and RNA. Regions in the DNA-RNA plane which correspond to the various cell cycle phases are shown. Cells to the right of the dashed line can be lysed by DNase and trypsin treatment, proving that they are dead. Note the higher proportion of dead tumor cells from mice treated with Aldomet as compared to controls.

on samples pretreated with DNase and trypsin to remove dead cells. No meaningful differences in the distributions of living tumor cells in the different stages of the cell cycle were observed when cells from Aldomet-treated and control mice were compared (Table 1).

In 3 additional experiments, mice were treated for 48 hr with Aldomet. The proportion of dead tumor cells and the cell cycle positions of living tumor cells were determined and compared to controls. The proportion of dead cells was again higher among tumor cells from Aldomet-treated mice than among tumor cells from control mice. Again, no differences in the proportions of cells in the various stages of the cell cycle were detected when living cells from experimental and from control mice were compared (Table 1).

DISCUSSION

Aldomet is toxic to C-1300 NB cells *in vivo*. Treatment with Aldomet of mice bearing C-1300 NB tumor for as brief a period as 24 hr kills a substantial proportion of the tumor cells. Tumor weight is not appreciably altered over this short time span but, in earlier work, we have shown that persistent treatment with Aldomet slows the growth of s.c. implanted C-1300 NB reproducibly and highly significantly (3).

Tumor cells teased into single cell suspension from solid tumors were analyzed by flow cytometry to determine their positions within the various stages of the cell cycle. After Aldomet treatment for either 24 or 48 hr, the proportions of living tumor cells in the G₁, S, or G₂ and M stages of the cell cycle were indistinguishable from proportions found among tumor cells from control mice. This finding suggests that the sensitivity of tumor cells to the drug, at least during the time periods studied, is unlikely to be related to the cell cycle.

We cannot offer any definitive explanation for our results, especially since the mechanisms of action of Aldomet are not fully understood. Several mechanisms of action of Aldomet are known, including dopa decarboxylation, false neurotransmission, and stimulation of α -adrenergic receptors (5, 7-9, 11). The Aldomet analogue L-dopa methyl ester is toxic not only to NB cells but also to other tumor cell lines *in vitro*; yet, *in vivo*, Aldomet suppresses growth of C-1300 NB selectively (3, 12). We postulate that Aldomet is taken up by NB cells because of the catecholaminergic metabolic pathway that they possess and that it exerts a direct toxic effect *in situ*.

Table 1
Cell cycle stages of C-1300 NB cells from Aldomet-treated and control mice

Cell cycle	Experiment	Control mice		Aldomet-treated mice			
		% of cells	Mean	24-hr treatment		48-hr treatment	
				% of cells	Mean	% of cells	Mean
G ₁	1	51.9		55.0		56.3	
	2	49.1	49.7 ± 1.6 ^a	47.0	49.7 ± 2.6	50.0	53.5 ± 1.9
	3	48.0		47.2		54.2	
S	1	28.9		25.2		20.6	
	2	31.9	31.8 ± 1.6	32.8	30.7 ± 2.6	29.0	26.1 ± 2.8
	3	34.6		34.2		28.8	
G ₂ + M	1	19.2		19.8		23.1	
	2	19.0	18.5 ± 0.57	20.2	19.5 ± 0.48	21.0	20.3 ± 1.8
	3	17.4		18.6		17.0	

^a Mean ± S.E.

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