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

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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

NB 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 a 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 treatment of newborn mice with nerve growth factor, which causes hypertrophy of the SNS, augments NB growth significantly (1, 4).

RESULTS

Tumors from 16 Aldomet-treated mice weighed 129 ± 36 (S.E.) mg. Tumors from 14 control mice weighed 186 ± 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 71.9 ± 2.9% (6 experiments); for tumors from control mice, it was 46.9 ± 1.8%. This difference is highly significant (p < 0.001). Flow cytometric analysis showed 68.4 ± 5.3% dead cells in tumor cell suspensions from Aldomet-treated mice (same 6 experiments); 50.0 ± 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
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 (F355.575) and RNA (F445-575) 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.

Table 1

| Cell cycle stages of C-1300 NB cells from Aldomet-treated and control mice |
|-----------------------------|-----------------------------|
|                             | Control mice                | Aldomet-treated mice       |
|                             | 24-hr treatment | 48-hr treatment | 24-hr treatment | 48-hr treatment |
| Cell cycle                   | % of cells | Mean            | % of cells | Mean            | % of cells | Mean            |
| G1                          |           |                 |           |                 |           |                 |
| 1                           | 51.9      | 49.7 ± 1.6a     | 55.0      | 49.7 ± 2.6      | 56.3      | 53.5 ± 1.9      |
| 2                           | 49.1      | 47.0            | 47.0      | 47.2            | 50.0      | 54.2            |
| 3                           | 48.0      | 28.9            | 25.2      | 20.6            |           |                 |
| S                           |           |                 |           |                 |           |                 |
| 1                           | 31.9      | 31.8 ± 1.6      | 32.8      | 30.7 ± 2.6      | 29.0      | 26.1 ± 2.8      |
| 2                           | 34.6      | 34.2            | 34.2      | 28.8            |           |                 |
| 3                           | 19.2      | 19.8            | 19.8      | 23.1            |           |                 |
| G2 + M                      |           |                 |           |                 |           |                 |
| 1                           | 19.0      | 18.5 ± 0.57     | 20.2      | 19.5 ± 0.48     | 21.0      | 20.3 ± 1.8      |
| 2                           | 17.4      | 18.6            |           |                 | 17.0      |                 |

*Mean ± S.E.

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).

Toxicity of Aldomet to Mouse Neuroblastoma in Vivo 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).

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.
REFERENCES


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