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The cytolytic effect of L-2,4 diaminobutyric acid with malignant glioma cells and fibroblasts*

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Summary. L-2,4-Diaminobutyric acid (DABA), an amino acid analogue, produced a cytolytic effect with a human glioma cell line, SKMG-1, and normal human fibroblasts. The concentrations of DABA necessary to reduce the cell count to 50% of control (LD_{50}) following a 24-h incubation at 37°C were 12.5 m*M* for the human fibroblasts and 20 m*M* for the glioma cell line. The concentrations of DABA necessary to produce an LD_{50} after a 48-h incubation at 37°C were 10 m*M* for the human fibroblasts and 14 m*M* for the human glioma cell line. The cytolytic effect of DABA was similar in the absence or the presence of serum with the human glioma cell line. The cytolytic effect of 20 m*M* DABA was partially prevented by the presence of 5 m*M* methyl-AIB. DABA was not preferentially toxic to this human glioma cell line compared with normal fibroblasts.

Introduction

L-2,4-Diaminobutyric acid (DABA), an amino acid analogue, is transported into the synaptosomal fraction isolated from rat brain by the GABA carrier [3]. DABA accumulates extensively in Ehrlich cells against a steep concentration gradient [2].

DABA uptake into Ehrlich cells via the A neutral amino acid transport system was not saturable and resulted in cellular damage [1]. In addition, DABA induced complete cellular lysis with a human glioma cell line U-178MG at 6m*M* and with a rat glioma cell line BT5C at 20 m*M* during a 24-h incubation at 37°C. Furthermore, there was no evidence of cellular lysis under identical conditions with normal human glial cells, even at a 20 m*M* concentration of DABA. The cytolytic effect of DABA on these cells was prevented by 2-(methylamino)-isobutyric acid (methyl-AIB), a specific amino acid analogue for the A neutral amino acid transport system [4].

In order to determine whether a selective cytolytic activity of DABA could be demonstrated with another tumor cell line as against normal cells, we examined the effect of this compound on a human glioma cell line and a normal fibroblast strain.

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Methodology

Materials. DABA and methyl-AIB were purchased from Sigma. Gentamicin was purchased from Schering (Pointe Claire, Quebec). McCoy's 5A medium and fetal calf serum (FCS) were purchased from GIBCO (Burlington, Ontario).

Cell culture. The human glioma cell line, SKMG-1, was obtained from Dr Greg Cairncross (London, Ontario). The human glioma cell line was maintained in McCoy's 5A medium plus 10% FCS and gentamicin (8 μg/ml). The doubling time was approximately 12 h. For experiments, the cells were trypsinized and seeded into 60-mm petri dishes at an initial concentration of 300 000 cells/dish. The dishes were then incubated at 37°C for 24 h in 5% CO_2. For experiments in the absence of serum, the medium was removed, the dishes were washed twice with the above McCoy's 5A medium without serum, and they were then incubated with the same medium. For experiments in the presence of serum, the medium was not changed. DABA, methyl-AIB or NaCl was added to appropriate dishes. The dishes were incubated for an additional 24–48 h. At 24 and 48 h after the addition of the above compounds, the medium was removed and the dishes were washed twice with the appropriate medium. The cells were detached by trypsinization and enumerated in a model F_n Coulter Counter. The data are expressed as a percentage of control values (control dishes were incubated with 20 m*M* NaCl). There was no difference in the growth of cells in the presence or absence of NaCl. The experiments in the presence of serum were done twice and those in the absence of serum, three times.

The human fibroblast cell strain, DHF, which was established from the foreskin of a normal 9-year-old boy, was obtained from Dr Ralph Germinario (Lady Davis Institute, Montreal, Quebec). The fibroblast strain was utilized during the 10th to 12th passages. The doubling time was approximately 66 h. Experiments were done just as described above, except that no experiments without serum were done. The experiment was done twice. The data are presented as means ± SE.

Results

Figure 1 shows the sensitivity of the human glioma cell line, SKMG-1, and of the normal fibroblast cells following a 24-h incubation in the presence of 10% FCS and

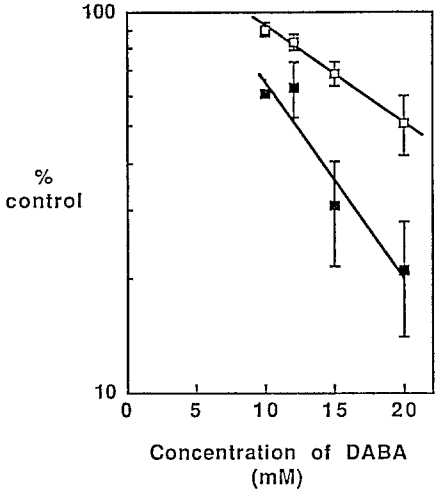

Fig. 1. Cytolytic effects of DABA in the human glioma cell line SKMG-1 (□) and in the normal fibroblast cell strain DHF (■) following a 24 h incubation with increasing concentrations of DABA in McCoy's 5A media with 10% FCS at 37°C. The data is expressed as the mean ± SE of the percent of control where control samples were incubated with 20 m*M* NaCl

graded concentrations of DABA. The concentrations of DABA necessary to result in 50% of control were 12.5 m*M* for the human fibroblast cells and 20 m*M* for the glioma cell line.

Figure 2 shows the sensitivity of the human glioma cell line and of the normal fibroblast cells following a 48-h incubation in the presence of 10% FCS and graded concentrations of DABA. The concentrations of DABA necessary for 50% of control were 10 m*M* for the fibroblast cells and 14 m*M* for the human glioma cell line. The survival curve for the human glioma cell line in the absence of serum and the presence of graded concentrations of DABA was not significantly different from that obtained in the presence of serum (data not shown).

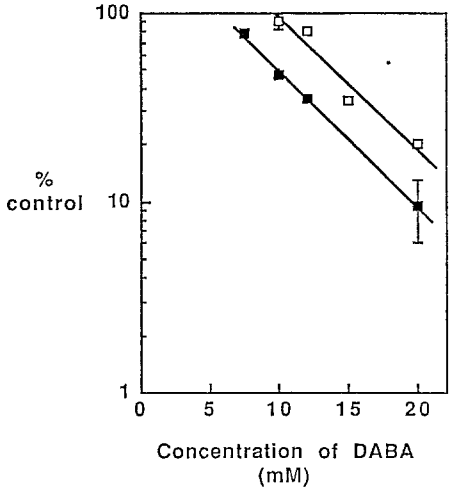

Fig. 2. Cytolytic effects of DABA in the human glioma cell line SKMG-1 (□) and in the normal fibroblast cell strain DHF (■) following a 48 h incubation with increasing concentrations of DABA in McCoy's 5A media with 10% FCS at 37°C. The data is expressed as the mean ± SE of the percent of control where control samples were incubated with 20 m*M* NaCl

Table 1. Effect of methyl AIB on the cytolytic action of DABA following a 48 hour incubation in the presence of 10% FCS

Cell type	Conc. DABA (mM)	Conc. methyl AIB (mM)	% Control (Mean ± SE)
Human glioma cell line	–	5	89 ± 1
Human glioma cell line	20	–	20 ± 1
Human glioma cell line	20	5	63 ± 1
Human fibroblasts	–	5	96 ± 12
Human fibroblasts	20	–	10 ± 4
Human fibroblasts	20	5	44 ± 4

Table 1 demonstrates that 5 m*M* methyl-AIB was able to partially prevent the cytolytic effect produced by 20 m*M* DABA in the human glioma cell line and human fibroblasts.

Discussion

The results of this study confirm the cytolytic effect of DABA on human cells. This effect is not secondary to a nonspecific osmotic effect, since neither 20 m*M* NaCl nor 20 m*M* methyl-AIB significantly altered the growth of these cells. As previously noted, the cytolytic effect of DABA may be secondary to an intracellular hyperosmolar state resulting in cell lysis. The intracellular hyperosmolar state appears to be secondary to the unlimited uptake of DABA by the A neutral amino acid transport system [1, 4]. This would thus explain the protective effect of methyl-AIB [4].

We were not able to show a preferential cytolytic effect of DABA on the human glioma cell line as compared with the normal fibroblasts, in contrast to the results of Ronquist et al. [4]. This discrepancy may be secondary to (a) heterogeneity of normal human cells to the cytolytic effects of DABA, i.e., normal glial cells are more resistant than fibroblasts, and (b) heterogeneity of human glioma cell lines to the cytolytic effect of DABA, i.e., the SKMG-1 glioma cell line is more resistant to the cytolytic effects of DABA than the U-178MG line. Further investigations with other normal human cells, such as hematopoietic precursor cells, and other tumor cell lines should help to better define the selectivity of DABA for malignant cells.

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