Effect of DL-Glyceraldehyde on Mouse Neuroblastoma Cells in Culture

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SUMMARY

Neuroblastoma cells had a much higher rate of glycolysis than the Chinese hamster ovary K1 cells, although the former cell type had a longer doubling time (24 hr) than did the latter (12 hr). DL-Glyceraldehyde inhibited the glycolysis in both cell types to the same extent, but it reduced the growth of neuroblastoma cells more markedly than that of Chinese hamster ovary K1 cells. These data indicated that glycolysis may be more critical for the growth of neuroblastoma cells than for the Chinese hamster ovary K1. In our experimental conditions the higher glycolysis rate did not correlate with the faster growth rate of cells in culture.

INTRODUCTION

DL-Glyceraldehyde, a potent glycolytic inhibitor (10, 22), reduces the growth of many types of rodent neoplasms (2, 3, 7, 19, 21). These results appear to be in agreement with Warburg's hypothesis that the high rate of anaerobic glycolysis of tumors in vitro is a unique requirement for neoplastic growth. However, there is evidence that this metabolic anomaly is not due to a fundamental difference between normal and tumor cells but merely reflects their particular growth rate (1, 6, 8, 9, 12, 23). We report that neuroblastoma cells have a much higher rate of glycolysis than CHO-K1 cells, although the former cells have a longer doubling time than the latter. Although DL-glyceraldehyde inhibited the glycolysis in both cell types to the same extent, it reduced the growth of neuroblastoma cells more markedly than that of Chinese hamster ovary K1 cells. Since the doubling time of neuroblastoma cells (24 hr) was 2-fold longer than that of CHO-K1 cells. Thus, under our experimental conditions, the higher rate of glycolysis does not correlate with the faster growth rate of cells in culture.

MATERIALS AND METHODS

Cell Culture and Drug Treatment. In our laboratory, mouse NB cells have been in culture for more than 1 year. Cells were grown as a monolayer in Falcon plastic flasks containing F12 medium with 10% gamma globulin newborn calf serum, penicillin and streptomycin (100 μg/ml and 100 units/ml) and were maintained at 36° in a humidified atmosphere of 5% CO2 in air. Cells have not been cloned. When grown in Falcon plastic flasks, the average doubling time of NB cells is about 24 hr. Our NB cell line contains acetylcholinesterase but no butyrylcholinesterase (17). Tyrosine hydroxylase is present in the primary tumor (4, 18), but it is barely detectable in the long-term culture; however, the enzyme activity is restored to the initial level in the dibutyryl cyclic 3', 5'-AMP-induced differentiated cells (K. N. Prasad, J. Waymire, and N. Weiner, in preparation). CHO-K1 cells were grown under experimental conditions identical to those of NB cells and had a doubling time of 12 to 13 hr. DL-Glyceraldehyde was dissolved in F12 medium without serum immediately before use and added to NB cell culture at a concentration of 0.01 to 1.0 mM. Control cells were treated similarly, except that no drug was added.

Measurement of Survival, Growth, and Differentiation. The growth of cell population was determined by counting with a hematocytometer or Coulter counter the total cell number as a function of time after treatment. For a single cell suspension, cells were incubated in 0.25% Viokase solution for 20 min. The cells that stained with trypan blue (0.1% in NaCl solution) were considered dead and were subtracted from the total counts. Since the percentage of dead cells in control and drug-treated populations was always less than 1%, the counting in the subsequent experiments was done in a Coulter counter. The number of cells in control and drug-treated cell population was determined, and growth inhibition 1, 2, and 3 days after drug treatment was calculated as follows:

\[
\frac{\text{No. of control cells} - \text{No. of drug-treated cells}}{\text{No. of control cells}} \times 100
\]

A cytoplasmic extension greater than 50 μm in length was considered to be an axon, and axon formation was regarded as an expression of morphological differentiation (16).

Measurement of Lactate. The amount of lactate in the medium of neuroblastoma and CHO-K1 cells was determined at the Pediatric Microchemistry Laboratory of University of Colorado School of Medicine, Denver, Col., by measuring the generations of NADH at 340 nm (11). The final cell density at the time of lactate measurement was similar in both cell types. The blank (medium without cells) was prepared similarly.

RESULTS AND DISCUSSION

Table 1 shows that neuroblastoma cells had a much higher rate of glycolysis than the CHO-K1 cells. Since the doubling time of neuroblastoma cells (24 hr) was 2-fold longer than the CHO-K1 cells (12 hr), the higher rate of glycolysis in these cell
Table 1

Lactate production in the neuroblastoma and CHO-K1 cells

Neuroblastoma ($10^4$ cells) and CHO-K1 ($5 \times 10^4$ cells) were plated in Falcon plastic dishes (60 mm), and DL-glyceraldehyde (1.0 mM) was added 3 days after plating. After 4 hr of incubation, the drug was removed, and the fresh growth medium was added. Control cells were treated similarly, except that no drug was added. Lactate was determined 20 hr after the removal of glyceraldehyde. The final cellular density of control neuroblastoma and CHO-K1 cells at the time of lactate measurement was similar. Each value represented an average of 3 samples.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Treatments</th>
<th>Lactate/liter (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroblastoma</td>
<td>Control</td>
<td>6.28 ± 0.60$^a$</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>Glyceraldehyde</td>
<td>4.76 ± 0.51</td>
</tr>
<tr>
<td>CHO-K1</td>
<td>Control</td>
<td>2.67 ± 0.20</td>
</tr>
<tr>
<td>CHO-K1</td>
<td>Glyceraldehyde</td>
<td>1.79 ± 0.21</td>
</tr>
</tbody>
</table>

$^a$ Mean ± S.D.

Chart 1. Neuroblastoma or CHO-K1 cells were treated with DL-glyceraldehyde (1 mM) 24 hr after plating. The percentage of inhibition as a function 2 days after the addition of drug was calculated as follows:

$$\left(\frac{\text{No. of control cells} - \text{no. of drug-treated cells}}{\text{no. of control cells}}\right) \times 100$$

Each value represented an average of at least 6 samples. Vertical bars, S. D. S. D.'s of points not shown in the figure were approximately equal to the size of the symbol.

The percentage of inhibition of population growth was dependent upon drug concentration and on length of exposure. Chart 2 shows that a treatment period of 10 hr produced 88% inhibition of neuroblastoma cell growth. On removal of drug (1 mM) and readdition of fresh growth medium 4 hr after treatment, the cell number and the viability 1 day later did not change. However, the drug-treated cells during the 2nd and 3rd days of treatment grew with the same doubling time as those of controls. This is substantiated by the fact that the percentage of growth inhibition 2 and 3 days after drug treatment was similar. Thus, glyceraldehyde induces inhibition of cell division which is reversible after the removal of drug. In the drug-treated cell population, the tendency of cells to pile on top of each other was similar to that of controls.

Although DL-glyceraldehyde inhibited the cell division of neuroblastoma cells, it did not induce morphological differentiation. It has been reported (13) that dopamine and 6-hydroxydopamine induce inhibition of cell multiplication without any morphological differentiation. The present study further supports the previous conclusion (15) that the inhibition of cell division is not sufficient for induction of morphological differentiation. On the contrary, after treatment with dibutylryl cyclic 3', 5'-AMP (16, 17) and prostaglandin E1 (14), the inhibition of cell multiplication is secondary to the induction of morphological differentiation.

Pyruvic acid reverses the inhibition of glycolysis by glyceraldehyde in tumor slices (6, 10) but not in brain slices (5, 20). In order to study the effect of pyruvic acid on glyceraldehyde-induced inhibition of neuroblastoma cell growth, DL-glyceraldehyde (1 mM) and pyruvic acid (1 mM) were added to neuroblastoma cell culture simultaneously. After 4 hr of incubation, cells were washed twice, and fresh
growth medium was added. Growth inhibition was measured 2 days after glyceraldehyde treatment. Results of this study demonstrated that pyruvic acid did not reverse the effect of DL-glyceraldehyde on mouse neuroblastoma cells. This is consistent with the study on brain slices (5, 20). Pyruvic acid by itself had no effect on the growth of neuroblastoma cells in vitro. From the present study, the following conclusions may be drawn: (a) a higher rate glycolysis did not reflect a faster growth rate, because CHO-K1 cells, which have shorter doubling time than the neuroblastoma cells, produce lesser amount of lactic acid; and (b) although DL-glyceraldehyde inhibits glycolysis to a similar extent in both cell types, it reduced the growth of neuroblastoma cells more markedly than that of CHO-K1 cells. Thus, anaerobic glycolysis may be more important for the growth of neuroblastoma cells than for the CHO-K1 cells.

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REFERENCES

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