ABSTRACT

Hematopoietic and nonhematopoietic cell lines cultured in media containing either 5-methyltetrahydrofolate or 5-formyltetrahydrofolate grow well to the same extent. However, when these same cell lines are grown in the presence of nitrous oxide, selective growth inhibition can be shown for hematopoietic cells cultured in 5-methyltetrahydrofolate-containing media. These cells also demonstrated a decreased ability to suppress [3H]-thymidine incorporation into DNA in deoxyuridine suppression tests.

INTRODUCTION

Since the 1950s, N2O, an anesthetic agent, has been known to cause megaloblastic changes in human hematopoietic cells (14). Two groups reported that N2O suppressed the proliferation of leukemia cells from the patients with acute and chronic myelogenous leukemia (10, 15). For a long time, however, the mechanisms of action of N2O on such cells were unknown. Recently, it turned out that N2O inactivates 5-methyl-THF-homocysteine methyltransferase (8, 12) by oxidizing the vitamin B12 from the Cob(I)alamin to the Cob(III)alamin form (2, 3). Inactivation of this enzyme prevents the conversion of 5-methyl-THF to tetrahydrofolate. Tetrahydrofolate is converted to 5,10-methyltetrahydrofolate, and the latter is required for DNA synthesis.

Human bone marrow cells treated with N2O show the impairment of DNA synthesis and an increased number of cells in the early S phase of DNA synthesis (1, 6, 12). These observations suggest a theoretical role for N2O as a cancer-therapeutic agent. The effect of N2O on the growth of human cells has not been investigated in depth.

Therefore, we studied the effect of N2O on the growth of various kinds of human cell lines using the dUrd suppression test, a good marker for defining biochemical megaloblastosis due to deficiency of folate and vitamin B12 (7). Our data indicate that cell lines derived from hematopoietic cells are more sensitive to N2O than are cell lines derived from nonhematopoietic cells and that the dUrd suppression test values are abnormal in such N2O-sensitive cells.

MATERIALS AND METHODS

Materials. [3H]dThd was purchased from the Radiochemical Centre, Amersham, England. Hanks' balanced salt solution and modified McCoy's Medium 5A, without folate acid and vitamin B12, were purchased from Nissui, Tokyo, Japan. Fetal calf serum was purchased from Flow Laboratories, Rockville, Md. Other chemicals obtained were: dUrd and sodium ascorbate (Wako, Tokyo, Japan); 5-methyl-THF (Sigma Chemical Co., St. Louis, Mo.); 5-formyl-THF (Lederle, Tokyo, Japan); and methylcobalamin (Eizai, Tokyo, Japan).

Cell Lines. The human cell lines used were K562 (Philadelphia chromosome positive chronic myelogenous leukemia) (17); HL-60 (promyelocytic leukemia) (5); TALL (T-cell acute lymphocytic leukemia) (19); Raji (Burkitt's lymphoma) (22); RPMI 1788 (B-cell line derived from normal volunteer); NCTC 2544 (normal fibroblast); HMV 143 (melanoma) (13); and Okajima (stomach carcinoma). The Okajima cell line was established and kindly supplied by Masanori Shimoyama of the National Cancer Center, Tokyo, Japan. RPMI 1788 and NCTC 2544 were purchased from Flow Laboratories, Rockville, Md.

Cell Culture. All the cell lines were grown in modified McCoy's Medium 5A supplemented with 20% fetal calf serum, gentamicin (60 mg/liter), methylcobalamin (5 [mu]g/liter), and 2 x 10^-7 M 5-methyl-THF.

[1] This work was partly supported by a Grant-in-aid from the Japanese Ministry of Education.
[2] To whom requests for reprints should be addressed.
[3] Present address: Third Department of Internal Medicine, Tokyo University, Hongo, Tokyo, Japan.
[4] The abbreviations used are: 5-methyl-THF, 5-methyltetrahydrofolate; dUrd, deoxyuridine; dThd, thymidine; 5-formyl-THF, 5-formyltetrahydrofolate.

Received March 1, 1982; accepted December 10, 1982.

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RESULTS

Effect of N₂O on Cell Growth. Chart 1, A to E and Table 1 show the effects of N₂O on the cell growth. As shown in Chart 1A, in the absence of N₂O, K562 cultured in media containing either 5-formyl-THF or 5-methyl-THF grew at a nearly identical rate. However, in the presence of N₂O, K562 cells cultured in media containing 5-methyl-THF were not able to grow, while cells cultured in media containing 5-formyl-THF grew as well as those cultured in the absence of N₂O. A similar trend was observed for HL-60 (Chart 1B), T-ALL, Raji, and RPMI 1788 (Chart 1C).

In contrast, NCTC 2544 (Chart 1D) cultured in the media containing either 5-formyl-THF or 5-methyl-THF were capable of growth in both the presence and the absence of N₂O. Similar phenomena were observed in HMV 143 (Chart 1E) and Okajima (Chart 1F) cell lines.

Effect of N₂O on dUrd Suppression Test. Chart 2, A to E, and Table 2 show the effect of N₂O on the dUrd suppression test. Chart 2A shows the dUrd suppression test value of K562. In the absence of N₂O, cells cultured in the media containing either 5-formyl-THF or 5-methyl-THF showed the ability to suppress [3H]dThd incorporation into DNA at similar rates when dUrd is added. In the presence of N₂O, cells cultured in the media containing 5-methyl-THF, but not 5-formyl-THF, showed a decreased ability to suppress the [3H]dThd incorporation into DNA.

Table 1

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Absence of N₂O</th>
<th>Presence of N₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>+5-formyl-THF</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+5-methyl-THF</td>
</tr>
<tr>
<td>Hematopoietic cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K562</td>
<td>1.4</td>
<td>8.5 ± 0.2</td>
</tr>
<tr>
<td>HL-60</td>
<td>1.1</td>
<td>6.0 ± 0.2</td>
</tr>
<tr>
<td>T-ALL</td>
<td>1.2</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>Raji</td>
<td>1.2</td>
<td>8.0 ± 0.2</td>
</tr>
<tr>
<td>RPMI 1788</td>
<td>1.5</td>
<td>8.3 ± 0.4</td>
</tr>
<tr>
<td>Nonhematopoietic cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCTC 2544</td>
<td>90</td>
<td>305 ± 17</td>
</tr>
<tr>
<td>HMV 143</td>
<td>50</td>
<td>350 ± 13</td>
</tr>
<tr>
<td>Okajima</td>
<td>0.7</td>
<td>2.7 ± 0.1</td>
</tr>
</tbody>
</table>

*Mean ± S.E.

mg protein/bottle.
Effects of Nitrous Oxide on Human Cell Lines

Chart 2. Effect of N2O dUrd suppression test values of human cell lines in media containing 5-methyl-THF (5-CH3THF) or 5-formyl-THF (5-CHOThF). A, K562; B, HL 60; C, RPMI 1788; D, NCTC 2544; E, HMV 143; 2F, Okajima.

Table 2

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Absence of N2O</th>
<th>Presence of N2O</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+5-formyl-THF</td>
<td>+5-methyl-THF</td>
</tr>
<tr>
<td>Hematopoietic cells</td>
<td>K562</td>
<td>10.7 ± 0.9*</td>
</tr>
<tr>
<td></td>
<td>HL-60</td>
<td>14.6 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>T-ALL</td>
<td>21.3 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Raji</td>
<td>18.9 ± 4.8</td>
</tr>
<tr>
<td></td>
<td>RPMI 1788</td>
<td>23.4 ± 1.5</td>
</tr>
<tr>
<td>Nonhematopoietic cells</td>
<td>NCTC 2544</td>
<td>23.0 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>HMV 143</td>
<td>22.2 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>Okajima</td>
<td>22.7 ± 1.8</td>
</tr>
</tbody>
</table>

* 0.1 µmol/ml.
* Mean ± S.E.

at dUrd concentrations of 0.01, 0.1, and 1.0 µmol/ml. At dUrd concentrations of either 0.01 or 0.1 µmol/ml, similar tendencies were observed in HL-60 (Chart 2B), T-ALL, Raji, and RPMI 1788 (Chart 2C) cell lines. At higher concentrations of dUrd, obvious differences in the 4 groups were not always demonstrable (Chart 2C). In contrast, N2O had almost no effect on the dUrd suppression test values of NCTC 2544 (Chart 2D), HMV 143 (Chart 2E), and Okajima (Chart 2F) cell lines.

DISCUSSION

The present work has demonstrated that N2O inhibits the proliferation of some human cell lines in culture media containing 5-methyl-THF. These results suggest that N2O may be effective in inhibiting the growth of certain tumors in vivo, since 5-methyl-THF is the predominant form of folate derivatives in plasma and tissue. Under the conditions we used, all the hematopoietic cells had suppressed growth in the presence of N2O. In contrast, growth of one benign cell line derived from epidermis and 2 malignant cell lines derived from nonhematopoietic cells was less affected in the presence of N2O.

There have been several reports concerning the effects of N2O on malignant cells. N2O suppressed the growth of leukemic cells from patients with chronic and acute myelogenous leukemia (10, 15). In transplant-treated mice, N2O had a slightly inhibitory effect of the growth of Ehrlich ascites tumor cells and fibrosarcoma cells and a remarkable inhibitory effect on the growth of lymphoid cells (4, 11, 20). However, N2O had no inhibitory effect on the...
growth of 2146 tumor (mouse ascites tumor) and M808 tumor (mouse mammary adenocarcinoma) (9, 20). In view of these reports and the data obtained in the present study and although the conditions used for the experiments were different, it is suggested that malignant cells derived from hematopoietic cells are more sensitive to the inhibitory effects of N2O than are those derived from nonhematopoietic cells.

It has been shown that N2O inactivates vitamin B12 through oxidation of Cob(I)alamin to Cob(III)alamin form (2, 3) and inhibits 5-methyl-THF-homocysteine methyltransferase activity (8, 12). We also studied cell growth in culture media containing 5-formyl-THF instead of 5-methyl-THF; 5-formyl-THF had a protective effect on cell growth which was suppressed in the media containing 5-methyl-THF. These results suggest that the inhibition of cell growth by N2O is attributable to the impairment of 5-methyl-THF metabolism through inhibition of cellular 5-methyl-THF-homocysteine methyltransferase activity.

We simultaneously carried out the dUrd suppression test on those cell lines which were incubated for 24 hr under various conditions. At dUrd concentrations of either 0.01 or 0.1 μmol/ml, the dUrd suppression test values correlated well with the results of cell growth. Cell growth which was suppressed by N2O showed a decreased ability to suppress the [3H]dThd incorporation into DNA. If N2O is used clinically for cancer patients, it is theoretically possible that the dUrd suppression test can be used in the dUrd suppression test values correlated well with the results of cell growth. When higher concentrations of dUrd, such as 1.0 and 10.0 μmol/ml, were used in the dUrd suppression test, test values were not always correlated with cell growth. This may be due to the fact that higher concentrations of dUrd not only may increase the dTTP pool but may also inhibit thymidine kinase directly (21).

In conclusion, our results indicate that N2O impairs the growth of hematopoietic cell lines through impaired 5-methyl-THF metabolism. In such cells, the dUrd suppression test shows decreased [3H]dThd incorporation into DNA and can, therefore, be used as a marker of N2O sensitivity.

ACKNOWLEDGMENTS

We are grateful to Keiko Kikumoto for her technical assistance and also to Dr. Rita Weiss, Department of Neoplastic Diseases, Mount Sinai School of Medicine, for the preparation of this manuscript.

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