The Effect of Chemotherapeutic Agents on the Incorporation of DNA Precursors by Experimental Brain Tumors

William R. Shapiro

Laboratory of Neurological Oncology, Sloan-Kettering Institute for Cancer Research, and Department of Neurology, Cornell University Medical College, New York, New York 10021

SUMMARY

A test system is described for chemotherapy of experimental brain tumors based on the effects of the drugs on the uptake and incorporation of DNA precursors. Thirteen to 15 days following intracerebral implantation of murine gliomas, the mice were given chemotherapeutic agents, following which $^3$H-labeled thymidine (Tdr-3H) or deoxyuridine-2-$^14$C (Udr-14C) was administered i.p. The effect of the chemotherapeutic agent on the incorporation into newly formed tumor DNA (and frequently that of normal tissues) of the DNA precursors was determined and compared with uptake in a control group of animals not treated with the agent. 1,3-Bis(2-chloroethyl)-1-nitrosourea significantly retarded the uptake of Tdr-3H into all three tumors tested. Uptake was retarded from 25 to 50%, depending on the dose of 1,3-bis(2-chloroethyl)-1-nitrosourea. 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea similarly produced retardation in uptake of Tdr-3H to approximately 40% of control. Depression began 12 hr after administration of the CCNU and was still evident 72 hr later. Arabinosylcytosine produced marked depression of uptake of Tdr-3H at 3 doses tested. At 40 mg/kg, uptake was retarded to 5% of control in the tumor (Glioma 261) and to 0 in lymph node and bone marrow; at doses of 400 and 4000 mg/kg, uptake was retarded to 0 in all tissues tested. Uptake into tumor of Tdr-3H was markedly enhanced by methotrexate with maximum effect occurring approximately 30 min after administration of the drug. A combined experiment with Udr-14C confirmed enhancement of uptake of Tdr-3H, while Udr-14C uptake was markedly retarded. Thirty min after the methotrexate, Tdr-3H incorporation increased to 140% of control; Udr-14C uptake diminished to 23% of control. In murine bone marrow, methotrexate had no effect on uptake of thymidine but produced a prompt fall in uptake of Udr-14C to 9% of control. This test system permits evaluation of a chemotherapeutic effect in experimental brain tumors, examines possible mechanisms of drug action, and permits inferences on blood-brain barrier function.

INTRODUCTION

This report describes a test system for chemotherapy of experimental brain tumors based on the effects of drugs on the uptake and incorporation of precursors into tumor DNA. The test system attempts to fulfill the following aims: (a) to establish a system for preliminary testing of chemotherapeutic agents in experimental brain tumor chemotherapy; (b) to examine possible mechanisms of drug action; and (c) to measure ability of drugs to enter tumor cells within the substance of the brain.

The test system is based on the carcinogen-induced murine ependymoblastoma model (2). Previous reports described the use of the brain tumor model to test chemotherapeutic agents for possible use in patients with malignant brain tumors (20–22). In the model, fragments of s.c.-carried tumors are implanted into the brains of mice; the animals are treated with chemotherapeutic agents and survival is determined. This survival model demonstrated several useful features. First, it was based on a tumor which was induced in brain tissue. Chemotherapeutic agents were thus tested against brain tumors per se, possibly an important characteristic of a model designed to test agents for human brain tumor chemotherapy. Second, tumors were implanted into the brains of animals, permitting the testing of agents in the natural milieu of the brain tumor. The model was thus distinguished from those previously used in which brain tumor tissue implanted in extracerebral sites was used. Third, the model was able to differentiate among chemotherapeutic agents by a method which permitted statistical evaluation. The model thus demonstrated that the nitrosoureas BCNU3 and CCNU were highly effective in increasing survival time of tumor-bearing animals while other drugs, e.g., MTX, were ineffective.

Although such features represented improvements over other models available at the time, some limitations were still evident. Evaluation of agents was time consuming and utilized hundreds of animals. Because the average duration of survival of brain tumor-bearing animals ranged from 20 to 30 days, 2 to 6 months were required before an agent could be considered useful or ineffective. Thus, the first aim of the current study was to develop a system in which a possible

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3The abbreviations used are: BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; MTX, methotrexate; i.e., intracerebral; Tdr-3H, thymidine-$^3$H; ara-C, 1-β-D-arabinofuranosylcytosine, arabinosylcytosine, cytosine arabinoside; Udr-14C, deoxyuridine-2-$^14$C; EpA, ependymoblastoma A; TCA, trichloroacetic acid.
therapeutic result could be obtained within 1 month on a relatively small number of animals. A drug that demonstrated retardation in uptake of DNA precursors could then be taken through more complicated survival studies, while drugs that failed to show an effect on DNA precursor incorporation would be set aside from further study.

The initial test system, because it was based on survival time, gave no indication as to possible mechanisms of action of the agents used. Demonstration of an altered uptake and incorporation of DNA precursors might yield clues to possible mechanisms of drug action. Thus, a reduction in incorporation of DNA precursors would imply a reduction in the total available synthesizing cell pool, e.g., by reduction of DNA synthesis or by alteration in the fraction of cells in S phase. The second aim of this study, then, was to examine such mechanisms.

The survival model showed that MTX was ineffective in prolonging the survival of mice bearing i.c. gliomas (22). With the extensive literature demonstrating the inability of MTX to penetrate the blood-brain barrier, it was thought that MTX might be ineffective because of its failure to gain entry into the tumor. The 3rd aim of the present study was to see whether MTX altered the incorporation of DNA precursors, thus demonstrating conclusively that it could enter brain tumor cells.

With these aims in mind, 4 drugs were tested in the current system. BCNU was highly effective in prolonging survival time of glioma-bearing mice (22). The effect of BCNU on the incorporation of DNA precursors into the brain tumor model would thus serve as the basic test drug for the system. The results showed that BCNU significantly retarded the uptake of TdR-3H into all 3 tumors tested and thus clearly established that the system did detect an intracranial event. The 2nd drug tested was the closely related nitrosourea CCNU, which increases survival time in the murine glioma model when administered both as a suspension i.p. (20) and when given i.m. dissolved in sesame oil (21). Since the nitrosoureas demonstrate delayed toxicity, the time course of their action with respect to uptake of DNA precursors is of interest. The results demonstrated that CCNU retarded uptake of TdR-3H by 12 hr after administration; such reduced incorporation was still evident 72 hr after the drug was given. ara-C had not previously been tested as an agent against the murine gliomas, but, as reported here, the drug modestly increased survival time. As an example of a cell cycle-specific agent, ara-C demonstrated prompt and major retardation in uptake of TdR-3H. Finally, MTX was chosen to test its entry into brain tumor cells. MTX increased the uptake of TdR-3H while retarding the uptake of UdR-14C, confirming that the drug does enter i.c. tumors.

**MATERIALS AND METHODS**

**Brain Tumors.** Details of the model methodology have been reported elsewhere (2, 22). C57BL/6J male mice weighing 20 to 24 g were used. Of the original 4 tumor lines carried by s.c. transplantation (2), 3 were used in the present series of experiments: Glioma 261 was originally induced by Seligman and Shear (19); Glioma 26 was induced by Sugiuira (24), and EpA was a mutant subline of the ependymoblastoma of Zimmerman and Arnold (28). For i.c. implantation, 12- to 14-day-old s.c. tumors were cut into 1-mm fragments and the fragment was mounted in the tip of a spinal needle to which a metal guard had been fitted. The tip of the needle was inserted through the scalp and skull to a constant depth in mice under light anesthesia. The tumor fragment was extruded by the use of the obturator of the needle. The fragment was implanted into the right cerebral hemisphere about 2 mm below the surface of the brain. For the drug studies reported here, animals were used between 13 and 15 days after tumor implantation.

**Isotopes.** TdR-3H was purchased from Schwarz-Mann, Orangeburg, N. Y., as thymidine-methyl-3H at a specific activity of 1.9 Ci/m mole. UdR-14C was purchased from Amersham/Searle Corp., Arlington Heights, Ill., at a specific activity of 39.4 mCi/m mole. The nucleosides were made up in sterile water and injected i.p. with a Hamilton syringe and PB600 repeating dispenser. TdR-3H was administered at a dose of 40 µCi/mouse; UdR-14C was administered at a dose of 4 µCi/mouse.

**DNA Extraction.** Sterile trypan blue 1% 0.3 ml was given i.p. 4 to 16 hr prior to sacrifice in order to delineate the tumor from the surrounding unstained brain. Two hr after the injection of TdR-3H, the animals were placed under light ether anesthesia and sacrificed by cervical transection. The brain was removed and the tumor was dissected free. In some experiments, specimens of brain distant from the tumor, lymph node, and bone marrow were also obtained. Lymph nodes were dissected from each axilla and bone marrow was obtained from the 2 femora by aspiration or by flushing with cold 0.9% NaCl solution. All samples were immediately frozen in Dry Ice. DNA was extracted by a modification of the methods of Mizuno and Humphrey (14) as follows. Tissues were homogenized in iced 10% TCA and centrifuged, and the precipitate was washed in iced 5% TCA. RNA was hydrolyzed by adding 0.3 N KOH and heating to 37° for 60 min, reprecipitating the remainder in 10% TCA. Finally, the DNA was hydrolyzed by adding 5% TCA and heating at 90° for 60 min. The samples were centrifuged at room temperature and the supernatants were decanted into tubes for further analysis.

DNA was analyzed by the method of Webb and Levy (27). To duplicate 2-ml aliquots of unknowns and DNA standards were added 2 ml of 5% TCA. DNA for the standards was supplied as calf thymus DNA and obtained from Calbiochem, Los Angeles, Calif. p-Nitrophenylhydrazine reagent (purchased from Eastman Kodak, Rochester, N. Y., and prepared daily as 0.5% in ethanol), 0.2 ml, was added and the samples were heated in a 100°C water bath for 20 min. For removal of the excess p-nitrophenylhydrazine reagent, 10 ml of n-butyl acetate were added to each tube; the tubes were covered and vigorously shaken for 5 min and centrifuged, and the organic layer was decanted. Three ml of the aqueous layer were transferred to a 5-ml volumetric flask; 1 ml of 0.2 N NaOH was added; the fluid was diluted to volume with distilled water, mixed, and read against a distilled water blank in a Beckman DU spectrophotometer at 560 nm. All steps were done in duplicate, and the readings were converted to µg of DNA/ml by means of a standard curve. This method of DNA analysis differed from the standard Dische method which uses...
diphenylamine (18). According to Webb and Levy, the \( p \)-nitrophenylhydrazine method is more sensitive than that of Dische. The standard curve included samples of DNA ranging from 5 to 150 \( \mu \)g/ml. Blanks generally read 0.003 to 0.010 A and the 5 \( \mu \)g/ml DNA standard read approximately 0.027 A. DNA values in the unknowns below 5 \( \mu \)g/ml were discarded.

For determination of the amount of radioactive tracer in the samples, 0.2 ml of the DNA-containing hydrolysate was transferred to tared counting vials and weighed, and 0.3 ml of NCS solubilizer was added. Twenty ml of a scintillation cocktail consisting, per liter, of PPO (3 g), POPOP (100 mg), methyl alcohol (250 ml), and toluene, 750 ml, were added to the counting vials. Counting was done with a Packard Tri-Carb scintillation spectrometer (Packard Instrument Co., Inc., Downer's Grove, Ill.) equipped with an automatic activity analyzer which corrected for quenching in the samples. TdR-\(^3\)H was counted to <1.5% (S.D.) counting error; UdR-\(^4\)C was counted to no more than a 5% error and most of the samples were counted to less than 2.5% error. The counts (dpm/ml) were divided by the \( \mu \)g/ml of DNA and the result expressed as dpm/\( \mu \)g DNA. When deemed useful, the absolute uptake of thymidine in \( \mu \)g could be directly calculated based on the specific activity of the administered nucleoside.

**Drug Studies.** BCNU and CCNU were supplied by the Cancer Chemotherapy National Service Center. ara-C was purchased commercially as Cytosar from the Upjohn Company, Kalamazoo, Mich. MTX was purchased from Lederle Laboratory Division, American Cyanamid Company, Pearl River, N. Y. BCNU was dissolved in 2.5 ml of absolute ethanol, to which were then added 7.5 ml of 0.9% NaCl solution, and was administered i.p. (0.01 ml/g of body weight). CCNU is insoluble in aqueous media; in previous studies, it had been suspended and administered i.p. A method for dissolving the drug in sesame oil and administering it i.m. has recently been found to give results equal to or better than that of the i.p. suspension (21). Since such a method may be applicable to clinical chemotherapy, we decided to use it in these experiments. The drug was given in a dose of oil equal to 0.005 ml/g of body weight, ara-C and MTX were diluted with sterile water and administered i.p. at doses to yield 0.01 ml of drug solution per g of body weight of the animal.

The dose of each drug was chosen either from toxicity data obtained previously in this laboratory or from the literature. Time of drug administration relative to the DNA precursors varied with each agent, and dose and time are noted under “Results.” In each drug experiment, a minimum of 10 animals served as a control for the diluent, and there were 10 treated animals at each of the dose levels or at each time interval. After administration of the DNA precursor, the animals were sacrificed by alternating control and treated animals.

**RESULTS**

In the results that follow, the data have been organized under the headings of the various drugs. However, in the actual experiments, 2 drugs were frequently combined with a single control group of animals, e.g., BCNU and MTX were combined on several occasions in a TdR-\(^3\)H experiment. In each of the drug studies reported below, the Roman numeral of the original experiment is included, to identify common controls.

**BCNU.** On the basis of the finding of Mizuno and Humphrey (14) that the maximum effect of BCNU occurs 24 hr after its administration, the drug was given i.p. to animals bearing each of the 3 tumor lines 24 hr prior to the administration of TdR-\(^3\)H. The results are shown in Table 1. In previous studies, the 10% lethal dose for BCNU was 34 mg/kg (22). In EpA, 2 doses were used, 1 at 30 mg/kg and 1 at 40 mg/kg. In tumor lines Gloma 261 and Gloma 26, a single dose of 40 mg/kg was used. Significant depression of uptake and incorporation of TdR-\(^3\)H occurred in all 3 test systems. Within control and experimental groups, the coefficient of variation ranged from 20 to 30%. The control values, however, differed considerably between experiments, both in the BCNU studies and those presented below for the other drugs. For this reason, control groups were always included, and results were expressed both as dpm/\( \mu \)g and as “percentage of control.” The causes for such variation may be multiple and include differences in the size of the tumor particle inoculated, variation in the degree of necrosis within the tumors, differences in the percentage of cells in active DNA synthesis, differences in the percentage of cells in active DNA synthesis, the degree of illness of the mice at the time of the experiment, and alterations in overall and specifically tumor blood flow. Such differences in control values preclude any but rough comparisons of results between experiments. Since the intent

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Experiment</th>
<th>BCNU dose (mg/kg)</th>
<th>Incorporation DNA (dpm/( \mu )g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EpA</td>
<td>XI</td>
<td>30</td>
<td>157 ± 6.72 (10)</td>
</tr>
<tr>
<td>EpA</td>
<td>XIV</td>
<td>40</td>
<td>326 ± 18.0 (7)</td>
</tr>
<tr>
<td>Gloma 261</td>
<td>XIII</td>
<td>40</td>
<td>243 ± 22.9 (10)</td>
</tr>
<tr>
<td>Glioma 26</td>
<td>XVI</td>
<td>40</td>
<td>246 ± 24.0 (10)</td>
</tr>
</tbody>
</table>

\(^6\)Student’s \( t \) test, unpaired samples.

\(^b\) Mean ± S.E.; numbers in parentheses, numbers of animals.
of the experiments was to demonstrate primarily a first approximation of the effects of drugs on DNA precursor incorporation in experimental brain tumors, no such comparison would be warranted.

CCNU. CCNU was administered i.m. to animals bearing i.c. EpA tumors in a dose of 60 mg/kg in sesame oil (21). Because the time course of action of CCNU was unknown, the drug was given to different groups of mice at intervals of 0, 12, 24, 48, and 72 hr prior to the administration of TdR-3H. Three control groups were given diluent (sesame oil) alone at 0, 24, and 72 hr prior to the TdR-3H. Sixty mg/kg is the approximate 10% lethal dose for CCNU in this system. The time course of action of CCNU was unknown, the drug was given to different groups of mice at intervals of 0, 4, 12, 24, and the groups were therefore combined. Significant retardation in uptake and incorporation of TdR-3H occurred when CCNU was administered 12, 24, 48, and 72 hr prior to the TdR-3H. The approximate 10% lethal dose for CCNU in this system. The control group consisted of 25 animals every 2 days for 10 doses. Modest but significant increases in life-span occurred at or over doses of 65 mg/kg/day. Lethality was not achieved in the doses used and, therefore, the maximum tolerated dose was not determined.

Mizuno and Humphrey (14) had demonstrated that ara-C had its greatest effect immediately after its administration; therefore, with i.c. Glioma 261-bearing animals every 2 days for 10 doses. Modest but significant increases in life-span occurred at or over doses of 65 mg/kg/day. Lethality was not achieved in the doses used and, therefore, the maximum tolerated dose was not determined.

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Table 2

<table>
<thead>
<tr>
<th>Drug dose (mg/kg/dose)</th>
<th>Median day of death (T/C)</th>
<th>p by Wilcoxon test</th>
<th>Median life-span % control</th>
</tr>
</thead>
<tbody>
<tr>
<td>39</td>
<td>28.7/27</td>
<td>N.S.</td>
<td>106</td>
</tr>
<tr>
<td>65</td>
<td>33.5/27</td>
<td>&lt;0.01</td>
<td>124</td>
</tr>
<tr>
<td>108</td>
<td>34/27</td>
<td>&lt;0.0001</td>
<td>130</td>
</tr>
<tr>
<td>180</td>
<td>35/27</td>
<td>&lt;0.00001</td>
<td>137</td>
</tr>
<tr>
<td>300</td>
<td>37/27</td>
<td>&lt;0.0001</td>
<td>137</td>
</tr>
</tbody>
</table>

*p < 0.0001.

Table 3

<table>
<thead>
<tr>
<th>Drug dose (mg/kg)</th>
<th>N</th>
<th>Tumor</th>
<th>Lymph node</th>
<th>Bone marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>10</td>
<td>577 ± 94.2a</td>
<td>104 ± 22.9</td>
<td>420 ± 48.8</td>
</tr>
<tr>
<td>40</td>
<td>10</td>
<td>26 ± 3.3</td>
<td>0</td>
<td>8.3</td>
</tr>
<tr>
<td>400</td>
<td>4</td>
<td>9.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4000</td>
<td>10</td>
<td>2.0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*a Mean ± S.E.

As a result of the ara-C administration at all dose levels tested. Differences from control are significant at all doses in all tissues; p < 0.001.
even when doses of 300 mg/kg/day were used, only a 37% increase in survival over control occurred on the every-2-day schedule (Table 2). More important, however, is the fact that the low dose of ara-C produced a detectable effect in the uptake experiments which would have led to further studies if this method were used to screen new agents.

**MTX.** As noted above, several experiments were done in which BCNU and MTX were compared in their effects on the uptake and incorporation of TdR-3H. Usually, deoxuryridine is used when studying MTX effect since MTX blocks the methylation of dUMP to dTMP. Under such circumstances, deoxyuridine uptake is depressed. Thymidine uptake might be expected to be increased by MTX since intracellular thymidine depletion produced by MTX would result in an increase in the amount of uptake of exogenous thymidine. Such a result was obtained in the present studies. Table 4 depicts the results of 3 experiments with MTX. With Glioma 26, there was a marked increase in uptake of TdR-3H, and this was confirmed with Glioma 261. A separate experiment with Glioma 261 in which MTX was given 30 min before the TdR-3H also produced a marked increase in uptake of nucleoside. For a more detailed investigation of the effects of MTX, the drug was given to animals bearing i.e. Glioma 261 simultaneously with or at 30 min and 120 min prior to the administration of TdR-3H. Samples of brain tumor, lymph node, and bone marrow were obtained and incorporation of TdR-3H was determined in 9 animals in the control group and 10 animals each in the 3 drug-treated groups. Chart 2 depicts the results. uptake being expressed as percentage of control; increased uptake occurred at all 3 time intervals within the tumor. No significant change occurred in the bone marrow. Lymph node uptake was increased when the drug and isotope were administered at the same time, but 30-min and 120-min sampling showed return of the uptake to control levels.

Finally, both TdR-3H (40 μCi/mouse) and UdR-14C (4 μCi/mouse) were given to animals bearing i.e. Glioma 26. MTX was administered 30 min prior to the injection of the DNA precursors. The results are shown in Table 5. As expected, there was a modest rise of TdR incorporation into the tumor; at the same time, there was a marked depression of uptake of UdR-14C with a fall to 23% of control. Changes in both the thymidine and the deoxyuridine uptake were significant. In the bone marrow, as previously demonstrated, no significant change occurred in thymidine uptake, but there was a prompt fall in uptake of UdR-14C to 9% of control. The difference between the uridine and thymidine results in the bone marrow is not explained but may be related to the availability of thymidine in the marrow pool.

**DISCUSSION**

The first aim of the test system was to design a method for preliminary testing of chemotherapeutic agents in experimental brain tumor chemotherapy. All 4 drugs tested, BCNU, CCNU, ara-C, and MTX, altered the uptake of DNA precursors

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**Table 4**

**Effect of MTX on the uptake and incorporation of TdR-3H by i.e. murine gliomas**

MTX, 60 mg/kg, was administered i.p. at time shown relative to TdR-3H.

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Experiment</th>
<th>MTX time</th>
<th>Incorporation (dpm/μg DNA)</th>
<th>% of control</th>
<th>p^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glioma 26</td>
<td>XVI</td>
<td>0^b</td>
<td>244 ± 35.8 (10)^c</td>
<td>445 ± 32.1 (8)</td>
<td>181 &lt;0.001</td>
</tr>
<tr>
<td>Glioma 261</td>
<td>XIII</td>
<td>0^b</td>
<td>170 ± 11.3 (9)</td>
<td>390 ± 29.1 (10)</td>
<td>229 &lt;0.001</td>
</tr>
<tr>
<td>Glioma 261</td>
<td>XVIII</td>
<td>30 min^d</td>
<td>231 ± 26.1 (6)</td>
<td>445 ± 84.3 (6)</td>
<td>193 &lt;0.05</td>
</tr>
</tbody>
</table>

^a Student's t test, unpaired samples.
^b The MTX and TdR-3H were given simultaneously at 0 time.
^c Mean ± S.E.; numbers in parentheses, number of animals.
^d The MTX was given 30 min prior to the TdR-3H.
into experimental brain tumors. The first 2 of these drugs had already demonstrated an ability to increase the survival time of such animals. ara-C had not been tested previously, but in the present report was found to retard markedly the uptake of TdR-3H into the experimental brain tumor. Had the uptake experiment been a preliminary one, subsequent experiments would have demonstrated the modest increase in survival also noted. MTX had been tested previously and found to be incapable of significantly increasing survival time of mice bearing i.e. gliomas. However, the drug altered uptake of DNA precursors, clearly excluding the blood-brain barrier as a cause for the poor results in the survival studies.

The test system had as its second aim to examine possible mechanisms of drug action. With respect to specific drug effects, the data yielded several important findings. The effect of BCNU on uptake and incorporation of DNA precursors into brain tumors was similar to that reported by Mizuno and Humphrey (14) who found a 24-hr delay in maximum effectiveness in L1210 and Sarcoma 180. The results also demonstrated a lag in the effect of CCNU on TdR-3H incorporation into murine gliomas; the major depression occurred between 0 and 12 hr. The reason for such delay has not been determined, but may be related to either possible conversion of the nitrosoureas to active metabolites or to the mechanism whereby the nitrosoureas retard DNA precursor incorporation (18).

Evidence that the nitrosoureas require conversion to an active metabolite is indirect and includes the fact that CCNU itself cannot be identified in the blood or urine of man after p.o. administration (17), and only at very low concentrations in blood or urine in animals after p.o. dosage (16). Even after i.p. injection, the amount of CCNU identifiable in brain, i.e. brain tumor and s.c. brain tumor in glioma-bearing mice is a very small proportion of the amount given (13). If conversion into an active metabolite occurs, the time course of such conversion might be slow enough to produce the delayed effect on thymidine incorporation.

The other possible explanation for such delay relates to the effect of the drugs on DNA synthesis. The nitrosoureas prolong the S phase of the mitotic cycle of several cell preparations (3) and inhibit the insertion of the C-8 position of the purine ring in L1210 ascites cells, suggesting a direct inhibition of DNA synthesis. However, uptake of precursors into DNA may also be altered by a change in the fraction of cells in S phase at a specific time after the administration of an agent. The delayed TdR-3H uptake might reflect an earlier drug action not directly related to DNA synthesis, but one which altered synthetic biochemistry by some other mechanism.

The effect of ara-C on uptake and incorporation of DNA precursors in the brain tumor was marked and immediate and suggested a direct effect on DNA synthesis. Its effect on DNA synthesis has been demonstrated in several systems (6, 7, 9, 11). The mechanism of such inhibition has not entirely been established, but may be related to a direct inhibitory action on DNA polymerase (7).

Finally, the test system attempted to infer ability of drugs to enter tumor cells within the substance of the brain. ara-C was early found to be effective against mouse leukemia L1210 even when the leukemia was located in the central nervous system (10). Dixon and Adamson (4) in 1965 reported that radioactive ara-C could be demonstrated in the cerebrospinal fluid in dogs following systemic administration. ara-C entry into solid brain tumor was confirmed by the results reported here.

In contrast to ara-C, MTX is clearly excluded by blood-brain barrier from normal brain and spinal fluid. In the previously reported drug studies based on survival of glioma-bearing mice, MTX was tested against all 3 of the brain tumors used in the present study (22). The schedule was a single injection every 4 days for 4 doses, and dosage ranged from 4 to 256 mg/kg/day. MTX was not statistically effective in prolonging survival of tumor-bearing mice, and it was thought that the drug might be ineffective because of its failure to gain entry into the tumor. A series of studies reported by Ausman and Levin (1) and by Levin et al. (12), however, demonstrated that MTX occupied approximately the same space within the murine ependymoblastoma as did inulin, a space close to 27% and considerably larger than the 1 to 2% space found in normal brain. These results demonstrated that MTX gained entry at

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Control (dpm/μg)</th>
<th>MTX (dpm/μg)</th>
<th>% of control</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TdR-3H</td>
<td>647 ± 64.0 (10)</td>
<td>903 ± 98.7 (10)</td>
<td>140</td>
<td>&lt;0.05</td>
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<tr>
<td>UdR-14C</td>
<td>28.7 ± 5.4 (10)</td>
<td>6.5 ± 0.96 (9)</td>
<td>23</td>
<td>&lt;0.005</td>
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<td>Bone marrow</td>
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</tr>
<tr>
<td>TdR-3H</td>
<td>376 ± 38.8 (10)</td>
<td>330 ± 27.3 (10)</td>
<td>88</td>
<td>N.S.</td>
</tr>
<tr>
<td>UdR-14C</td>
<td>11.8 ± 2.2 (10)</td>
<td>1.0 ± 0.29 (9)</td>
<td>9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
least to the extracellular space of the murine brain tumor. The next question was whether the drug entered the tumor cells themselves. Tator (25, 26) recently demonstrated by autoradiography the presence of MTX within experimental brain tumors but not normal brain. The most conclusive evidence that MTX can enter solid i.e. tumors, however, is afforded by the experiments reported here. MTX clearly altered uptake and incorporation of DNA precursors within the tumor proper; UdR-14C uptake was depressed and TdR-3H uptake was increased by a single injection of MTX. Thus, the fact that MTX can enter the cells of a solid brain tumor was proven by its effect on intracellular metabolism. The reason that MTX did not significantly increase survival of glioma-bearing mice remains unknown, but it is clearly not related to drug entry. As with ara-C, it is possible that the time of action of MTX with respect to sensitivity of the cells is such that the schedule of once a day every 4 days originally used may not have been optimum.

Finally, specific results testing individual chemotherapeutic agents serve to confirm the usefulness of examining the effect of such agents on the uptake and incorporation of DNA precursors into experimental brain tumors. Similar uptake techniques have been reported in several test systems and have led to the suggestion that such methods could entirely replace survival studies (15). The complexities of brain tumors would probably prohibit such replacement, but they clearly do permit such a system for preliminary studies.

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The Effect of Chemotherapeutic Agents on the Incorporation of DNA Precursors by Experimental Brain Tumors

William R. Shapiro


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