Scheduling of Arabinosylcytosine and 6-Thioguanine Therapy

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SUMMARY

Mice treated simultaneously with 1-B-D-arabinofuranosylcytosine (ara-C) and 6-thioguanine (6-TG) were protected from the lethal effects of 6-TG. No protection was afforded if ara-C was given 16 hr before the 6-TG. DNA synthesis in mouse bone marrow was immediately depressed to 3 to 12% of that of control in various experiments, after treatment with ara-C, 10 to 20 mg/kg; it then rebounded to 148 to 180% of control during the period 8 to 16 hr, and was below control at 24 hr. DNA synthesis in L1210 cells treated in vivo with ara-C was depressed to less than 1% of control and did not begin to recover until almost 16 hr. However, after a second dose of ara-C at 24 hr, DNA synthesis in L1210 cells recovered much more rapidly.

We studied mouse bone marrows and L1210 cells treated with 2'-deoxythioguanosine to determine the persistence of acid-soluble 6-TG nucleotides after a single dose. 6-TG nucleotides declined to low levels in 6 to 8 hr, exhibiting half-times of approximately 3 hr in both tissues.

INTRODUCTION

It has been established that ara-C is an inhibitor of DNA synthesis, probably as a result of its effects upon DNA polymerase (2). A considerable weight of evidence indicates that 6-TG and 6-TGdR are lethal to cells because of its incorporation into DNA in place of guanine (1, 4, 6). 6-TG is not incorporated into DNA in place of guanine (1, 4, 6). j3-TGdR represents a 6-TG derivative designed to bypass mechanisms of resistance to 6-TG (7) and, for the purposes of this study, 6-TGdR and 6-TG can be regarded as equivalent. Their toxicities are equal, on a molar basis, in mice (7). These metabolic effects of ara-C and 6-TGdR indicate that they should logically be used in combination, since cells not sensitive to ara-C would continue to make DNA and incorporate 6-TG. The time sequence of the therapy would be of utmost importance. Indeed, the combination of ara-C and 6-TG has been found by Gee et al. (3) and others to be effective in the treatment of acute leukemia patients. As pointed out earlier (8), this was an empirical choice. Some support for the use of ara-C with 6-TG was provided by the finding of Schmidt et al. (13) that simultaneous treatment with ara-C and 6-TG made mice tolerant to high doses of the 6-TG.

It has been shown that bone marrow is the normal tissue damaged by 6-TG (11, 12). Any manipulation of DNA synthesis in bone marrow and tumor that causes time sequences to differ could permit protection of bone marrow and optimization of tumor therapy with 6-TG or 6-TGdR. Our experiments on mouse bone marrow and an experimental tumor line, L1210, indicated that such a circumstance may indeed be useful.

MATERIALS AND METHODS

The mice used in the experiments were CDF females, 8 to 10 weeks old, obtained from Rawley Farms, Plymouth, Mass., and AKR X DBA/2 F, (hereafter called AKD2F) or C57BL/6 X A F, (hereafter called B6AF,) female mice obtained from the Roscoe B. Jackson Laboratory, Bar Harbor, Maine. ara-C and 6-TG were provided by Drug Research and Development, National Cancer Institute, Bethesda, Md. Tritiated thymidine, 19 Ci/m mole, was purchased from Schwarz/Mann, Orangeburg, N. Y. Radiosulfur was purchased from New England Nuclear, Boston, Mass. 6-TG and 6-TGdR were labeled with radiosulfur by exchange, as described earlier (5). They were dissolved in 0.9% NaCl solution at pH 8 for administration.

For each experiment, mice of the same age group and in a very close weight range (e.g., 18 to 19 g) were selected, and group members were given identical dosages. For studies on bone marrow incorporations, each mouse was given the specified drug and, at the indicated time, was killed by cervical dislocation. The long bones of the hind legs were rapidly removed. The ends of each bone were clipped and marrow was ejected into chilled 0.9% NaCl solution by injection of the solution at 1 end with a No. 22 needle. In measurements of DNA synthesis with TdR-3H, each mouse was given an i.p. injection of 20 μCi TdR-3H and was killed 30 min later. Those to be labeled with 6-TG-35S or 6-TGdR-35S were given a specified dosage of the drug and, at the indicated time, were killed by cervical dislocation. Analyses for nucleotides in bone marrow and LI210 cells were made on perchloric acid extracts chromatographed on Whatman No. 1 filter paper and autoradiographed.
Scheduling of ara-C and 6-TG Therapy

Whatman No. 3MM papers with 5% KH₂PO₄ (RF values for 6-TG,  β-TGdR, and TGdMP were 0.25, 0.50, and 0.78, respectively).

RESULTS

Data presented in Table 1 are representative of several experiments conducted with groups of 6 mice. In the example, female B6AF1 mice were used. In confirmation of the findings of Schmidt et al. (14), the simultaneous administration of ara-C with 6-TG protected mice from doses of 6-TG that would otherwise be lethal. In addition, the experiment illustrates that this can be accomplished with daily doses over a relatively prolonged period, not just with a single dose. It also illustrates that when a dose of ara-C was given 16 hr before 6-TG, it was not protective.

Chart 1 illustrates the effect of ara-C (at several dose levels) on DNA synthesis in mouse bone marrow, as monitored with TdR-3H. DNA synthesis in bone marrow of mice dosed daily with ara-C for 5 days recovered at the same rate on Day 5 as did that in mice dosed only once. This indicates that the effects of such an ara-C regimen were reversible. Chart 2 shows that very similar results were achieved with the 3 labeled precursors, TdR-³H, 6-TG-³S, and β-TGdR-³S. DNA synthesis was reduced to 3 to 12% of control levels in various experiments, as soon as marrows could be sampled (5 min) after the injection of ara-C. Recovery was achieved in 6 to 8 hr (somewhat delayed at higher ara-C doses). There was an increase in DNA synthesis to above control levels (148 to 180%) in the period between 8 and 16 hr, and DNA synthesis was somewhat below control levels (71%) at 24 hr. Thus, the biochemical results indicated that bone marrows on the schedule showing protection from 6-TG by ara-C were incorporating little 6-TG into DNA. Those given β-TGdR or 6-TG 16 hr after the administration of ara-C incorporated relatively large amounts of 6-TG into DNA. The biochemical results with bone marrow therefore correlate with the observed toxicity. Chart 3 presents the results for L1210 cells growing in vivo, and for Mecca lymphosarcoma, a tumor relatively unresponsive to ara-C. The initial dose of ara-C depressed DNA synthesis in L1210 cells to less than 1% of control. Recovery did not begin until about 16 hr and began to rise rapidly between 16 and 24 hr. However, when DNA synthesis in L1210 cells in vivo was monitored after a 2nd dose of ara-C 24 hr later, the recovery was more rapid and was nearly complete by 16 hr. The reason for this change is not apparent and is under further study. The length of time that nucleotides of 6-TG were present in the cells as potential precursors of DNA would presumably affect the results. Charts 4 and 5 present data for the analysis of bone marrow and L1210 cells, respectively, after single doses of β-TGdR-³S. In both instances, the level of acid-soluble 6-TG nucleotides declined, with a half-time of about 3 hr, and had reached relatively low levels by 6 to 8 hr.

Table 1

Toxicity of 6-TG and its modification by ara-C

Groups of 6 B6AF1 female mice, initially weighing 21 g each, were used. All mice in Groups 1 and 3 had died by Day 16, while those of Groups 2 and 4 had returned to their initial weights.

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<th>Group</th>
<th>Treatment</th>
<th>No. of deaths</th>
<th>Maximal wt. loss (%)</th>
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<tr>
<td>1</td>
<td>6-TG, 8 mg/kg, daily for 9 days</td>
<td>6</td>
<td>30</td>
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<tr>
<td>2</td>
<td>ara-C, 10 mg/kg, followed in 10 min by 6-TG, 8 mg/kg, daily for 9 days</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>ara-C, 10 mg/kg, followed in 16 hr by 6-TG, 8 mg/kg, daily for 9 days</td>
<td>6</td>
<td>33</td>
</tr>
<tr>
<td>4</td>
<td>ara-C, 20 mg/kg, followed in 10 min by 6-TG, 8 mg/kg, daily for 9 days</td>
<td>0</td>
<td>12</td>
</tr>
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Chart 3. DNA synthesis in L1210 cells in vivo after the i.p. injection of ara-C at 15 mg/kg. Points, analyses of cells from 3 mice. Each mouse received i.p. injections of 1 x 10^6 L1210 cells and, on Day 5, each mouse received ara-C and i.p. injections of 20 µCi TdR-3H 30 min before sacrifice. L1210 cells were grown in CDF, female mice. Mecca lymphosarcoma cells were grown in AKD2F, female mice. Times plotted are for intervals after ara-C injection. The experiment is exemplary of several experiments that gave essentially the same results.

Chart 4. Persistence of 6-TG nucleotides in mouse bone marrows after treatment with β-TGdR-31S. Points, analyses of bone marrows from 3 CDF, female mice after single i.p. doses of β-TGdR-31S (8.5 mg/kg) expressed as µg 6-TG present per mg DNA. Replicate analyses agreed within 5%.

DISCUSSION

It would appear that the excellent results achieved by Gee et al. (3) and others with simultaneous administration of ara-C and 6-TG result from a high cell kill by ara-C and from effects upon the ara-C-resistant component of the population by 6-TG. In ara-C-resistant cells, DNA synthesis would not be depressed, and the utilization of 6-TG for DNA synthesis would proceed. This schedule of simultaneous administration would also seem advantageous from the standpoint of protecting the bone marrow from 6-TG. However, Gee et al. (3) gave ara-C parenterally and gave 6-TG p.o. It has been shown that this is not pharmacologically a simultaneous administration, since blood levels of 6-TG peak at 6 to 8 hr after a p.o. dose in humans (10). Some preliminary analyses on bone marrows from humans given single doses of ara-C indicate that, in contrast to mice, DNA synthesis was 1st stimulated and then depressed (G. A. LePage and J. A. Gottlieb, unpublished results). Thus, schedules for combining ara-C and β-TGdR in humans may need to be different from those optimum for mice, and further studies of scheduling seem indicated.

Mecca lymphosarcoma, a tumor relatively unresponsive to ara-C, showed depression of DNA synthesis to a low level, upon treatment with ara-C, but DNA synthesis recovered rapidly and went much above control levels at 24 hr. Advantage could be taken of such an effect when scheduling therapy with β-TGdR.

REFERENCES

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