Differential Sensitivities of Five Rat Hepatoma Cell Lines to Anticancer Drugs

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ABSTRACT

Five permanent tumor cell lines derived originally from either a solid or an ascites biopsy of rat hepatoma exhibited differential sensitivities to bleomycin, Adriamycin, 1-β-D-arabinofuranosylcytosine, hydroxyurea, 1-trans-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea, and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea. The cells were least sensitive to hydroxyurea and 1-β-D-arabinofuranosylcytosine, with some cell lines being almost totally resistant to these drugs. However, from 25- to 700-fold differences in survival were obtained between cell lines treated with either bleomycin or Adriamycin.

INTRODUCTION

We have recently demonstrated that 4 permanent cell lines of human malignant melanoma, all isolated from a single biopsy sample, expressed different in vitro survival sensitivities to ara-C, BLEO, and 1,3-bis(2-chloroethyl)-1-nitrosourea. This differential sensitivity to anticancer drugs could result in treatment failure if it is also expressed in vivo. Through the use of such tumor model systems, information can be obtained that may be pertinent to the selection of the proper chemotherapeutic drug combinations for the control of tumors that express multiclonal properties.

A transplantable rat hepatoma established in vivo by Chang et al. in 1967 (6), by induction with 3'-methyl-4-dimethylaminoazobenzene has been used by our laboratory to establish several cell lines of this tumor for in vitro drug studies. The permanent cell lines of solid rat hepatoma were cloned from single cells isolated from a single biopsy sample of a solid hepatoma. The ascites hepatoma cell lines were also cloned from abdominal fluid of rats that had been previously given injections of the hepatoma cells. In all, 10 lines were cloned from a solid tumor and 11 from ascites fluid and have grown in vitro for more than 5 years. Seven to 10 days later, the colonies were plated for colony formation. Ten to 11 day old, the colonies were stained and counted. A cell was considered to have retained reproductive potential if it gave rise to a colony composed of 50 or more cells (1, 2). The experiments were performed at least 3 times and averaged survival values are reported in the charts.

RESULTS

Response to BLEO. All 5 hepatoma cell lines exhibited biphasic survival responses to BLEO, characterized by steep and shallow slopes of the survival curves. The shallow slope of the survival curve was obtained at higher doses in the resistant cell lines. The shallow slope of the survival curve was obtained at lower doses in the sensitive cell lines.

Materials and Methods

Growth Conditions. Monolayers of the hepatoma cell lines were grown in Ham's F-10 medium supplemented with 20% fetal calf serum in a 5% CO2-95% air incubator at 37°. The 2 solid tumor lines were designated as Ad 3-3 and Bd 5-3, and the ascites lines as L1-3, and Ln 2-3, and Lz 2-3. Under these conditions, the growth fractions range from 60 to 97%, the population-doubling times range from 15 to 19 hr, and the control plating efficiencies range from 70 to 88%.

Survival Studies. Exponentially growing cells were treated with increasing doses of the drugs. After treatment the monolayers of cells were washed with Puck's Solution A and trypsinized from the plates. Cell counts and dilutions were made and the cells were plated for colony formation. Seven to 10 days later, the colonies were stained and counted. A cell was considered to have retained reproductive potential if it gave rise to a colony composed of 50 or more cells (1, 2). The experiments were performed at least 3 times and averaged survival values are reported in the charts.

Drug Solutions. The drug solutions were always prepared immediately before use to ensure against loss of activity. They were dissolved first in the appropriate solvent, and then diluted to final treatment concentrations in medium.

1 Supported by NIH Grant 5 R01 CA 15397 (03) and CA 16663 (03).
2 The abbreviations used are: ara-C, 1-β-D-arabinofuranosylcytosine; BLEO, bleomycin; ADR, Adriamycin, HU, hydroxyurea; MeCCNU, 1-trans-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea; CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; LD₅₀, dose lethal to 50% of the population. Received October 11, 1977; accepted December 8, 1977.
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The responses to BLEO of these hepatoma cell lines were biphasic. In all cell lines, the LD₅₀ (number obtained by extrapolating back to the Y axis from the straight line portion of survival curve), and Dₜ of the sensitive slope of line Ln 2-3 were identical to those of the other two ascites derived lines; however, the Dₚ of the resistant slope was almost 3 times higher [8 µg/ml for 1 hr (Table 1)].

Response to ADR. As with BLEO, the survival responses of these hepatoma cell lines to ADR was also biphasic. In addition, lines Bd 5-3, L1-3, Lz 2-3, and Ln 2-3 had small shoulder regions on their survival curves with extrapolation numbers ranging from 1.5 to 2.5 (Table 1). It can be seen in Chart 3 that Ad 3-3 was the most sensitive of the lines derived from solid hepatomas. The Dₜ's for the sensitive and resistant slopes of Ad 3-3 were 0.3 and 2.2 µg/ml for 1 hr, respectively, with an LD₅₀ of 0.8 µg/ml (Table 1). The differences in sensitivity of the two solid hepatoma cell lines to ADR were observable at doses as low as 0.1 µg/ml. At 10 µg/ml there were 20 times more cells killed in the Ad 3-3 population than in line Bd 5-3.

Two of the cell lines derived from ascites hepatoma had very similar responses to ADR. Chart 4 and Table 1 show that the sensitivities of lines L1-3 and Lz 2-3 were almost identical. The LD₅₀, n (number obtained by extrapolating back to the Y axis from the straight line portion of survival curve), and Dₜ of the sensitive slope of line Ln 2-3 were identical to those of the other two ascites derived lines; however, the Dₚ of the resistant slope was almost 3 times higher [8 µg/ml for 1 hr (Table 1)].

Response to ara-C. The responses to ara-C by cell lines derived from ascites hepatoma are shown in Chart 5A. Only 20% of the cells are killed by 1-hr exposure to ara-C in the most sensitive line, Ln 2-3. The other two ascites-derived lines show less response, and line L1-3 is almost totally resistant. No further reduction in survival was observed at doses as high as 1000 µg/ml.

The responses of the 2 lines cloned from the solid tumor are shown in Chart 5B. Line Ad 3-3 was the most sensitive with the survival fraction being reduced to about 0.6 at doses of 50 µg/ml or greater. Survival in Bd 5-3 remained above 0.9. No additional cell killing was obtained in either cell line at doses up to 1000 µg/ml.

Response to HU. Survival decreases only slightly in all clones at doses up to 1000 µg/ml, but then plateaus at 70% of the starting value for all lines.
Response to MeCCNU and CCNU. The survival curves of all of the hepatoma lines treated with MeCCNU or CCNU are characterized by a shoulder region followed by an exponential decrease in survival (Charts 7 and 8). Only small differences are observable between $D_{50}$'s and $LD_{50}$'s to 85% at higher doses (Chart 6).
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The experiments on the clones were performed over a 1-year period and the variability between experiments on the same drug was small, suggesting that the clones were genetically stable during the test period.

DISCUSSION

Because the currently used anticancer drugs are generally not selective enough to cause total tumor eradication, we often find that the initially successful chemotherapy is followed by recurrence of tumor growth, possibly due to the development of drug resistance. Such expressed or acquired resistance within a tumor may be the result of the ability of a cell to repair drug-induced damage (4, 8) or because of the presence of a number of different clones of cells within the tumor, all with different drug sensitivities (1, 2). The differential drug sensitivity in these clones may be caused by: (a) alterations in the cell membranes or cell surface components of the tumor cells, which in turn interfere with the transport of drugs into the cells (5, 7, 9); (b) variability in the conversion of the anticancer drug to an "inactive" form (2); (c) inability of the cell to "activate" the drug (2); or (d) the presence of alternative biochemical pathways to bypass the drug-inhibited step (3, 10). These are just a few of the possible causes of differential drug sensitivities or resistant in tumor cells.

The data presented in this paper illustrate that sensitivity to a given agent is expressed differentially by various clones of tumor cells derived from single tumor samples. All cell lines were very sensitive to BLEO, MeCCNU, CCNU, and ADR; the lines were only slightly sensitive to HU and ara-C. However, some differential sensitivity was observed after treatment with all 6 drugs tested.

The differential sensitivity was expressed maximally among the cell lines treated with BLEO, ADR, and ara-C. There was a 700-fold difference in the survival fractions of cells treated with BLEO doses of 50 μg/ml or greater (Chart 2). Cells treated with 10 μg of ADR per ml for 1 hr expressed a 25-fold difference in survival values (Chart 3). The biphasic nature of these survival curves (having sensitive and resistant slopes) may be related to differences in cell cycle responses to the drugs as we have demonstrated in Chinese hamster ovary cells (1, 4). The greatest variability in the hepatoma survival responses to ara-C was observed between lines Ad 3-3 and Bd 5-3 (Chart 5). We know from our previous studies on human melanoma sensitivities to ara-C that those cell lines having the highest ara-C kinase activities were the most sensitive to ara-C. Perhaps the same phenomenon will be

Table 2

Characteristics of the survival curves of the hepatoma cell lines treated with CCNU or MeCCNU

<table>
<thead>
<tr>
<th>Cell line</th>
<th>CCNU (μg/ml for 1 hr)</th>
<th>MeCCNU (μg/ml)</th>
<th>LD90 (μg/ml)</th>
<th>ri</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad 3-3</td>
<td>0.18</td>
<td>0.18</td>
<td>0.53</td>
<td>0.59</td>
</tr>
<tr>
<td>Bd 5-3</td>
<td>0.15</td>
<td>0.14</td>
<td>0.64</td>
<td>0.57</td>
</tr>
<tr>
<td>L 1-3</td>
<td>0.13</td>
<td>0.2</td>
<td>0.61</td>
<td>0.62</td>
</tr>
<tr>
<td>Lz 2-3</td>
<td>0.16</td>
<td>0.14</td>
<td>0.56</td>
<td>0.55</td>
</tr>
<tr>
<td>Ln 2-3</td>
<td>0.13</td>
<td>0.14</td>
<td>0.61</td>
<td>0.55</td>
</tr>
</tbody>
</table>

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illustrated for these hepatoma clones when the kinase and deaminase activities are tested.

We do not yet know the reasons for the differential drug sensitivities expressed by these hepatoma lines. Our studies on this phenomenon are centered on 3 areas: differences in recovery from drug-induced damage, differences in cell surface components, and in the case of ara-C, whether there are kinase and deaminase differences between the cell lines.

It is important that the mechanisms of acquired or natural resistance of tumor cells to chemotherapy drugs be elucidated, and that the studies be expanded to a variety of human tumors. In this way it may be possible to identify single drugs, combinations of drugs, and schedules that would be more effective at overcoming one of the classical problems in tumor cell chemotherapy, that of choosing a drug regimen for the optimal killing effect on a mixed population of cells within a tumor.

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REFERENCES

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