Application of in Vivo and in Vitro Pharmacokinetics for Physiologically Relevant Drug Exposure in a Human Tumor Clonogenic Cell Assay

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ABSTRACT

The biological half-lives and decay rate constants under the conditions of a human brain tumor clonogenic cell assay were determined for six clinically used anticancer agents. The agents studied were: 1,3-bis(2-chloroethyl)-1-nitrosourea; 3-(2-chloroethyl)-3-nitrosourea-2-deoxy-D-glucopyranose; cis-diaminedichloroplatinum(II); 2,5-diaziridinyl-3,6-bis(carboethoxyamino)-1,4-benzoquinone; 4-dimethyllepidophytoxin-D-thyildene glucoside; and 9-hydroxy-2-N-methylepiperazinoquinone, and 3-(2-chloroethyl-3-nitrosoureido-2-deoxy-D-glucopyranose. In vivo decay of all six drugs was found to be according to first order kinetics. The half-lives of two drugs, namely, 1,3-bis(2-chloroethyl)-1-nitrosourea and 3-(2-chloroethyl)-3-nitrosourea-2-deoxy-D-glucopyranose under the human tumor clonogenic cell assay (HTCA) conditions were found to be similar to their terminal in vivo half-lives in humans. For the other drugs, however, there was a very large difference between their in vivo and in vitro pharmacokinetics. In the case of 2,5-diaziridinyl-3,6-bis(carboethoxyamino)-1,4-benzoquinone, we observed about an 80-fold difference between its in vitro half-life of 40.76 h and its in vivo terminal half-life of 0.52 h. We describe the principles upon which these data can be used to design clinically more relevant in vitro drug exposure protocols inHTCAs. Since, generally, tumor cells are exposed to drugs in the HTCA either continuously or for a specified duration, e.g., 1 or 2 h, we computed the initial in vitro drug concentrations to which tumor cells should be exposed such that the resulting in vitro (c x t) after a 2-h or a continuous exposure will be within clinically achievable levels. The application of these in vivo and in vitro pharmacokinetic principles will provide for more physiological testing of patient tumor cell sensitivity to anticancer drugs in the HTCA, and is likely to result in lower rates of false positive responses in clinical trials using clonogenic cell assays.

INTRODUCTION

HTCAs have been used to study tumor biology, to determine the sensitivities of tumors of patients to chemotherapeutic agents, and more recently to investigate the antitumor activity of new anticancer drugs. The optimum design of drug exposure protocols for performing clinically relevant drug sensitivity studies utilizing HTCA, however, requires not only knowledge of the clinical pharmacology and pharmacokinetics of the agents being tested, but also information on drug stability and cell kill kinetics under the HTCA conditions. Such information is necessary to ensure that tumor cells are exposed to drugs at concentrations and for durations that are not only clinically relevant, but also are appropriate with the pharmacology, cell cycle phase specificity, and route of administration of the given drugs. Because data on intratumoral drug concentrations and drug decay kinetics are generally not available the clinical pharmacokinetic parameters most frequently used to determine drug exposures in clonogenic cell assays have been the drug peak plasma concentration, the drug plasma terminal half-life, and the drug plasma (c x t), representing the integral of plasma drug levels over time. However, in order to use these data to compute in vitro drug exposure times and drug concentrations such that the resulting in vitro (c x t) would be within clinically achievable values, one needs to know both the pharmacokinetic model that fits the dynamics of in vitro drug concentration changes over time and the drug decay rate constants under the conditions of exposure of the cells to the drug in the assay. Unfortunately, there is only little quantitative data available on the chemical and/or biological stability of chemotherapeutic agents under HTCA conditions.

In this study, we used a modification of a previously described bioassay to determine the in vitro stabilities of six antitumor agents, as reflected by their in vitro biological decay rate constants and half-lives under the conditions of a brain tumor clonogenic cell assay. The initial drug concentrations used in the bioassay were those concentrations which demonstrated a significant in vitro cell kill in a cytotoxicity prescreen using the 9L rat gliosarcoma cell line. The results show that while for the nitrosoureas in vitro half-lives under the clonogenic cell assay conditions were within the range of the terminal half-lives observed in clinical pharmacokinetic studies, for the other drugs there was a very significant difference between their in vivo and in vitro half-lives. Based on our data, we make suggestions as to in vitro drug concentrations which in (a) a 2-h treatment, and (b) a continuous exposure (greater than 5 in vitro half-lives) will result in drug exposures within clinically achievable levels. Using the principles described in this paper and the drug decay data we present, other in vitro drug concentrations and exposure durations can be computed with which more clinically relevant in vitro drug exposures can be achieved for different methods of clinical drug administration, e.g., intraarterial or i.p.

MATERIALS AND METHODS

9L Gliosarcoma. The 9L tumor cell line is an N-methyl-N-nitrosourea-induced gliosarcoma of the Fisher 344 rat. It produces solid brain tumors in rats within 2 weeks of intracerebral injection. The cells used for this study were maintained by weekly in vitro passages of monolayer cultures in MEM supplemented with EBS and 15% FCS. Human Glioma SF 126. The human glioma cell line SF 126 was originated in our laboratory from a fresh tumor biopsy obtained by craniotomy from a patient bearing a grade IV astrocytoma. The cells were cryopreserved in MEM containing 10% dimethyl sulfoxide and 20% FCS. Prior to use in these studies, thawed cells were washed twice with medium and cultured as monolayers in T75 flasks containing MEM, EBS, and 15% FCS. The cells were characterized by their positive reactivity to a monoclonal antibody, GE 2, which recognizes a glioma-associated antigen (16), using previously described methods (17).
All cultures used in the experiments were screened for and shown to be free of *Mycoplasma* contamination.

Drugs. The drugs studied were provided by the following suppliers: BCNU and Cis-Pt (Bristol Myers, Syracuse, NY); CHZ, VM 26, and AZQ (Drug Development Branch, National Cancer Institute, Bethesda, MD); HME (Sanofi-Labaz, Bordeaux, France, and kindly made available for these studies by Dr. Victor Levin).

Stock solutions were prepared by dissolving the weighed amounts of drug in different solvents, depending upon the solubility of the drug. Subsequent dilutions were made with Hanks' balanced salt solution. All drug solutions were prepared just before use and were kept on ice prior to addition to the treatment flasks. The solvents used and the highest final concentrations in culture were as follows: BCNU (ethanol; 1:4,000), CHZ (dimethyl sulfoxide; 1:4,000), AZQ (N,N-dimethylacetamide; 1:10,000), HME acetate (H2O; 1:10,000), and Cis-Pt (MEM; 1:10,000). VM 26 was dissolved at 10 mg/ml in a solvent consisting of 150 mg benzyl alcohol, 300 mg N,N-dimethylacetamide, 2.5% purified polyethoxylated castor oil, 300 mg N,N-dimethylacetamide, sufficient maleic acid to achieve a pH of 5.1, and 4,715 g absolute ethanol. The final solvent concentration in culture was 1:100,000.

For each experiment a set of control flasks were set up which were treated with solvent only at the concentrations stated above. Cytotoxicity Prescreen. In order to determine the initial drug concentrations at which to expose cells for the bioassay, a cytotoxicity study was performed for each drug by using the 9L gliosarcoma cell line. Peak plasma drug concentrations as reported in clinical studies were used to determine the range of concentrations for this initial prescreening study which was performed as described below. Confluent monolayer cultures were trypsinized with 0.25% trypsin in 0.4% EDTA solution in Ca2+/Mg2+-free phosphate-buffered saline and seeded in triplicate T25 tissue culture flasks at cell numbers ranging between 50 and 5000 cells (depending on the expected cell kill). MEM supplemented with EBS, 20% FCS, and 5 x 10^6-x-irradiated (4000 rads) 9L cells were added, and the flasks were incubated at 37°C for 24 h in a humidified atmosphere of 5% CO2/air. Fifty /g of stock solutions of each drug, prepared as previously described, were added to each set of flasks, the flasks were incubated for another 24 h, and then rinsed and refed with fresh MEM containing 20% FCS. After 2 weeks at 37°C and 5% CO2/air, colonies were stained with a 3% crystal violet/methanol solution and counted under low power microscopy.

Bioassay. The dose at which a 1-3-log clonogenic cell kill of the 9L tumor cell line was achieved in the prescreen was used as the initial concentration in the bioassay, which was performed as follows: a set of 4 x 775 tissue culture flasks were set up, each containing 1.5 x 10^6 SF 126 human glioma cells and 1.5 x 10^7 heavily irradiated 9L cells in MEM containing 20% FCS. The flasks were incubated at 37°C and 5% CO2 in a humidified incubator for 24 h to allow the cells to attach to the culture flask surface. Stock drug solution was then added to each flask to yield the predetermined initial drug concentration. At various time points, triplicate 1.5-ml aliquots of the supernatant were removed and immediately filtered through 0.4- /xm sterile cellulose acetate filters (Millipore, Bedford, MA). The first 0.5 ml was discarded and the remaining 1 ml was added to T25 tissue culture flasks, each containing the following: 10^5-10^6 9L cells (depending upon the expected cell kill), 5 x 10^6 heavily x-irradiated 9L cells, and MEM supplemented with 20% FCS. The time points at which the drug solutions were aliquoted were determined in pilot studies to be 0-60 min (BCNU, CHZ, and VM 26); 0-9 h (Cis-Pt); and 0-24 h (AZQ and HME). After 24 h at 37°C and 5% CO2, these second sets of flasks were rinsed twice, fresh MEM containing 20% FCS was added to them, and they were incubated as previously described. Colonies were stained and counted after 2 weeks.

Simultaneously, a dose-response study was performed as described in the prescreening experiments, but using ranges of drug concentrations which included the initial concentrations used in the bioassay described above.

Evaluation of Bioassay Survival Curves. For each drug, the surviving fraction, S.F. (t) of 9L gliosarcoma clonogenic cells at each treatment time point, was computed by using the equation

\[
\text{S.F.}(t) = \frac{\text{CFE}(t)}{\text{CFE}(0)}
\]

whereby CFE(t) is the colony-forming efficiency of cells treated with supernatant removed at time t and CFE(0) is the colony-forming efficiency of untreated control cells. CFE is defined as:

\[
\text{CFE} = \frac{\text{No. of colonies formed}}{\text{no. of cells seeded}}
\]

The surviving fractions were plotted against the time points at which the supernatants were removed. Each S.F.(t) was subsequently converted to the corresponding drug concentration, C(t) by reading off from the simultaneously determined dose-response curve, the drug concentration necessary to achieve an equivalent reduction in survival in the 9L clonogenic system. The *in vitro* decay rate constant, k, was then calculated by using the equation for first order kinetics.

\[
-k \frac{dc}{dt} = kC(0)
\]

which upon integration gives:

\[
C(t) = C(0) \cdot e^{-kt}
\]

where C(t) is the drug concentration remaining in the flask after time t and C(0) is the initial drug concentration.

Rearranging Equation D and taking logs gives

\[
k = \ln[C(0)/C(t)]/t
\]

and at

\[
t_n = 0.693/k
\]

k was computed by linear regression analysis of the concentration-time curve obtained for each drug, and using Equation F, the half-life t_n of the drug under the treatment conditions was computed.

Computation of Concentrations for Clinically Relevant Drug Exposure. In order to determine the initial concentration to which tumor cells should be exposed such that the resulting *in vitro* (c x t) is equivalent to the clinically achievable (c x t), the trapezoidal approximation (18) was used:

\[
(c \times t) = \frac{1}{2}(C_0 + C_1)(t_1 - t_0)
\]

\[
+ \frac{1}{2}(C_1 + C_2)(t_2 - t_1) + \frac{1}{2}(C_{n-1} + C_n)(t_n - t_{n-1})
\]

where C0, C1, C2 . . . Cn are the drug concentrations remaining in culture after exposure times of t0, t1, t2 . . . . tn, respectively. Since we had previously determined the *in vitro* decay rate constant k, it is possible to compute the remaining drug concentration, C(t), in culture at any given time, t, by using Equation D.

Substituting the values in Equation G allows calculation of C(0), the initial drug concentration required to achieve the clinical (c x t) after *in vitro* exposure of the tumor cells for a given time, e.g., 2 h. For a continuous drug exposure (t = o), the approximation used for calculating C(0) is as follows:

\[
(c \times t) = C(0)/k
\]

and rearranging gives

\[
C(0) = k \cdot (c \times t)
\]

where k is the *in vitro* decay rate constant of the drug and C(0) is the initial concentration to which tumor cells would be exposed.

RESULTS

Cytotoxicity Prescreen. Fig. 1 illustrates the survival curve obtained with cis-platinum in the prescreening study performed on the 9L gliosarcoma cell line. This representative curve is typical of the survival curves for the other five drugs. The results obtained from the survival curves for all six drugs are summa-
The highest variation in the two concentrations was 30% for HME. As mentioned earlier, however, this discrepancy between the two HME concentrations was probably mainly a result of drug adsorption onto the surface of the tissue culture flask. Correcting for this adsorption, a variation of 9% was computed between theoretical and experimental HME concentrations.

**Drug Concentrations for Clinically Relevant in Vitro Drug Exposure.** Table 4 gives the computed drug concentrations required to achieve clinical (c x t) levels in the clonogenic cell assay after (a) a 2-h drug exposure, and (b) a continuous exposure. For BCNU and CHZ these two concentrations were very similar and were 25.2 and 24.6 μM (BCNU), and 30.2 and 29.1 μM (CHZ), respectively. In contrast, for HME, AZQ, Cis-Pt, and VM 26, there were very wide differences in the *in vitro* concentrations required to achieve clinically relevant (c x t) levels under the two exposure conditions stated above.

**DISCUSSION**

Several reports (5–10) have documented, both retrospectively and prospectively, that human tumor clonogenic cell assays are capable of predicting the response of an individual patient to cancer chemotherapy. For previously untreated ovarian cancer patients with good performance status, the assay has been reported to perform better than the best choice of chemotherapy by the physician (6, 9). Generally, however, these studies have shown that clinical response correlates better with *in vitro* resistance (greater than 90%) than for sensitivity (50–70%). Significant among the assay-related factors that could account for these relatively low levels of positive *in vivo-in vitro* correlation rates are the design of the *in vitro* drug exposure protocols, the method of determining *in vitro* sensitivity indices (19), and the criteria for establishing the cut-off index for *in vitro* sensitivity to be used in such correlations (20). Although Alberts et al. (21) have reviewed the application of pharmacological principles in HTCAs and have proposed the concept of a "cut off" concentration for determining *in vitro* drug sensitivity, it has not been possible, in most cases, to apply these principles in an optimized fashion to HTCAs. This is primarily because little (12, 13) and for some drugs, no quantitative data on *in vitro* drug-decay kinetics under HTCA conditions are available. In the absence of such information, most drug sensitivity studies with the HTCA have been performed simply by exposing tumor cells to peak plasma drug concentrations, or a fraction (5–10%) of it, continuously or for 1–2 h.

In this report, a bioassay was used to investigate the pharmacokinetics of six clinically used anticancer agents under the conditions of a human brain tumor clonogenic cell assay. For all the drugs investigated, *in vitro* decay was according to first order kinetics. Similar first order kinetics have been reported for the *in vitro* decay of both *cis*-platinum (22) and BCNU (23) in human plasma. Under the HTCA conditions used in this present study, the two nitrosoureas, BCNU and CHZ, demonstrated *in vitro* half-lives of 0.54 and 0.52 h, respectively. These values are relatively close to their respective *in vivo* terminal half-lives of 0.36 and 0.4 h, as determined in human clinical phase I trials (23, 24). Furthermore, for these two drugs, because of their relatively high *in vitro* decay-rate constants of 1.28 h⁻¹ (BCNU) and 1.33 h⁻¹ (CHZ) and correspondingly

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**Table 1: Prescreen cytotoxicity data on 9L gliosarcoma cell line**

<table>
<thead>
<tr>
<th>Drug</th>
<th>In vitro concentration (μM)</th>
<th>Surviving fraction of 9L cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZQ</td>
<td>1.0</td>
<td>1 x 10⁻³</td>
</tr>
<tr>
<td>Cis-Pt</td>
<td>3.0</td>
<td>3 x 10⁻²</td>
</tr>
<tr>
<td>VM 26</td>
<td>0.15</td>
<td>1 x 10⁻²</td>
</tr>
<tr>
<td>BCNU</td>
<td>30</td>
<td>4 x 10⁻²</td>
</tr>
<tr>
<td>CHZ</td>
<td>30</td>
<td>2.7 x 10⁻²</td>
</tr>
<tr>
<td>HME</td>
<td>10</td>
<td>2 x 10⁻³</td>
</tr>
</tbody>
</table>
short in vitro half-lives, in vitro drug concentrations required to achieve clinically relevant (c x t) levels were similar for both a 2-h and a continuous drug exposure. In contrast, for AZQ, Cis-Pt, VM 26, and HME, we showed in this study that wide differences existed between their decay kinetics in the HTCA and their published human clinical plasma pharmacokinetics (17, 23–29). The in vitro half-lives of all four drugs were much longer than their in vivo terminal half-lives. For example, Curt et al. (26) reported an in vivo plasma terminal half-life of 0.52 h for AZQ compared with a half-life of 40.76 h we measured under our HTCA conditions. The terminal plasma disappearance rate of AZQ is therefore, almost 80 times faster than the biological decay rate of the drug in the HTCA. This long half-life of AZQ determined by bioassay may reflect, in part, the
fact that hydrolysis of AZQ can result in opening of the aziridinyl ring to yield potentially cytotoxic intermediates (30). However, we believe this to occur minimally in our system at the chloride, phosphate, and glucose concentrations. At the pH of 7.2 in the assay, the rate of such hydrolytic ring opening will be relatively low; the $t_{1/2}$ for AZQ at pH 7.2 is about 80 h (30). Furthermore, if two or more major cytotoxic effects (due to parent drug and degradation products) were being assayed, then the resulting decay curves will be bi- or multiphasic, in contrast to the first order decay kinetics we observed for AZQ and the other drugs. Thus, for AZQ, HME, Cis-Pt, VM 26, and other agents with half-lives that are much longer in vitro than in vivo, exposure of cells in peak plasma drug concentrations (or even 5 or 10% of it) will result in in vitro $(c \times t)$ levels that are many times higher than those achievable clinically. As a result, there is an increased probability of a false positive response and a simultaneously higher probability of a true negative response, as has been consistently observed in in vivo-in vitro studies that have been performed in this manner. The findings in this report of extreme differences in the in vitro and in vivo pharmacokinetics of some anticancer drug suggest the need for similar studies to be performed for other agents that are tested in the HTCA for clinical purposes. However, it is important to recognize that the biological assay used in the present study, unlike the HPLC assays generally used in in vivo pharmacokinetic studies, does not necessarily always measure the parent drug. Indeed, in some cases one may be measuring both the drug and its intermediates if both of these have cytotoxic activity. However, for some agents, e.g., the chloroethylnitrosoureas (31), the concentration of the drug degradation product is proportional to that of the parent drug, and decay of the drug intermediate is the rate-limiting step in the decay of the parent drug. Theoretically, in such situations, as of course is also the case for a drug whose cytotoxic action is due to the parent drug directly, a bioassay will provide for a true measure of the parent drug decay. Furthermore, first order kinetics, as we observed for all six drugs, strongly suggests the existence of only one major cytotoxic species. If more than one major cytotoxic species were to be present, then because of the expected different decay rate constants of these species, a bi- or multiphasic decay curve would be obtained.

The good correlation, $r = 0.986$, we observed between the theoretical and the bioassay-determined drug concentrations suggests that for the six drugs, the bioassay as described here allows for reasonably accurate determination of drug concentrations, and may be used in the absence of more specific assays requiring HPLC and other more complex techniques. However, a study comparing drug decay as measured by HPLC and by bioassay needs to be performed to confirm this for individual drugs.

Because of the differences in complexity of different HTCA techniques, differences are likely to exist in drug-decay kinetics under the different assay conditions. Such assay-specific variation in in vitro drug pharmacokinetics may require that drug decay be determined for each HTCA technique and the values obtained be used to optimize drug exposure in that assay. This problem may become less significant as the HTCAs become better optimized and standardized.

INDs pose an additional problem since there are usually no human pharmacokinetic data available for these agents at the time of initial screening. In the National Cancer Institute-sponsored multilaboratory study on the application of the HTCA to IND screening (11), tumor cells were exposed to drugs continuously at a single arbitrary concentration of 10 $\mu$g/ml. Active agents were then subjected to a dose-response study in order to better define their 70% infective dose values. This approach did not address any potential differences in the in vitro stability of the drugs. It may be advantageous even at this early stage of IND screening, using a simple bioassay such as we describe here, to determine the in vitro decay kinetics of agents that may be going into clinical trials. The data thus obtained could be used with clinical pharmacokinetic information that will be acquired during phase I clinical trials of active agents to better design further clinical trials of the agents with the HTCA.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Initial concentration (µM)</th>
<th>Half-life (h)</th>
<th>Decay rate constant (h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZQ</td>
<td>1.0</td>
<td>40.8</td>
<td>0.017</td>
</tr>
<tr>
<td>Cis-Pt</td>
<td>3.0</td>
<td>15.1</td>
<td>1.046</td>
</tr>
<tr>
<td>VM 26</td>
<td>0.15</td>
<td>36.5</td>
<td>1.019</td>
</tr>
<tr>
<td>BCNU</td>
<td>3.0</td>
<td>0.54</td>
<td>1.28</td>
</tr>
<tr>
<td>CHZ</td>
<td>3.0</td>
<td>0.52</td>
<td>1.33</td>
</tr>
<tr>
<td>HME</td>
<td>10</td>
<td>86.3</td>
<td>0.008</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mean peak plasma concentration (µM)</th>
<th>Termi- nal half-life (h)</th>
<th>$(c \times t)$</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>AZQ</td>
<td>10</td>
<td>1.9</td>
<td>0.52</td>
<td>10.4</td>
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<tr>
<td>Cis-Pt</td>
<td>100</td>
<td>8.3</td>
<td>15.07</td>
<td>6.5</td>
</tr>
<tr>
<td>VM 26</td>
<td>67</td>
<td>36.3</td>
<td>24.75</td>
<td>173.6</td>
</tr>
<tr>
<td>BCNU</td>
<td>220</td>
<td>9.2</td>
<td>0.36</td>
<td>19.2</td>
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<tr>
<td>CHZ</td>
<td>120</td>
<td>127.8</td>
<td>0.4</td>
<td>21.9</td>
</tr>
<tr>
<td>HME</td>
<td>2 mg/kg</td>
<td>2.5</td>
<td>4.4</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Theoretical Drug Conc. (µM)
PHARMACOKINETICS FOR DRUG EXPOSURE IN HTCA

In this present report, the clinical pharmacokinetic data used to perform the computations were all derived from published clinical phases I and II trials, in which drug administration was done i.v. The peak plasma drug levels, the achievable (c × t), exposure of cells as proposed in this paper is the cell cycle cols for these various clinical situations. Another important factor that must be considered when designing in vitro drug exposure of cells as proposed in this paper is the cell cycle phase specificity of the drugs to be tested. Kinetically, human tumors and consequently the cell suspensions prepared from them are asynchronous and only a fraction of the cell population will be in a given cell cycle phase at a given time (39). Consequently, exposure of the cells to agents such as vincristine, hydroxyurea, or 1-β-D-arabinofuranosylecytosine, which are cell cycle phase-specific in their mechanisms of action, will have to be for a duration of at least one cell cycle for any potential cytotoxic effect of the drugs to be observed. However, since generally cell cycle kinetic data are not available at the time of H, the drug in human subjects will provide for a theoretically theoretically cell cycle sensitivity assessed by in vitro continuous exposure to drugs. Cancer Res., 43: 2346–2349, 1983.


REFERENCES


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