Evaluation of Cell-killing Effects of 1-β-D-Arabinofuranosylcytosine and Daunorubicin by a New Computer-controlled in Vitro Pharmacokinetic Simulation System

Shinji Kishi, Nobuyuki Goto, Toru Nakamura, and Takanori Ueda

First Department of Internal Medicine [S. K., T. N., T. U.] and Department of Hospital Pharmacy [N. G.], Fukui Medical University, Matsuoka, Fukui 910-1193, Japan

ABSTRACT

An in vitro pharmacokinetic simulation system that can simulate plasma pharmacokinetics was established to evaluate the cytotoxicity of two representative antileukemic agents, 1-β-D-arabinofuranosylcytosine (ara-C) and daunorubicin. With this system, the survival rate of the cell line K562 treated with ara-C, relative to that of untreated control cells, was 71%, as determined by a clonogenic culture technique using a clinically intermediate dose of ara-C (1.0 g/m²; 2-h infusion). When the area under the serum concentration-time curve (AUC) was kept constant when the drug exposure times were 0.5, 2, 4, and 8 h, respectively, with a constant C × T, the relative survival rates [maximal concentrations (Cmax)] were 77.4% (0.40 μM), 11.6% (0.12 μM), 68.3% (0.06 μM), and 92.0% (0.30 μM). The conventional culture system, the relative survival rates (Cmax) following daunorubicin treatment were 7.6% (0.48 μM), 18.6% (0.12 μM), 63.7% (0.06 μM), and 92.0% (0.03 μM) when the drug exposure times were 0.5, 2, 4, and 8 h, respectively, with a constant C × T. When the drug concentration for 90% cell killing by the conventional culture system was plotted against the exposure time on a logarithmic scale, the regression line for daunorubicin had a slope of −0.40, whereas the slope of cis-diaminedichloroplatinum (or cisplatin), a typical AUC-dependent drug, was −0.98. These results suggested that daunorubicin was Cmax dependent rather than AUC dependent. In the simulation system, this Cmax dependency was apparently reduced, probably because of the smaller difference of Cmax in the simulation system compared with the conventional system with the constant AUC. Thus, this simulation system can predict the effects of ara-C, daunorubicin, and other antineoplastic agents much more exactly than the conventional culture system in clinical use.

INTRODUCTION

The in vitro tests used to evaluate the cytotoxic effects of antineoplastic agents do not necessarily reflect an agent’s actual clinical efficiency. One of the main reasons for this is that, in vitro, tumor cells are exposed to a constant concentration of a drug over a given period of incubation, whereas in the clinical use of these drugs (in vivo), the concentration of drug surrounding the tumor cells changes, based on the drug’s pharmacokinetic characteristics, including tissue distribution, metabolism, and excretion (1, 2). It is thus very important to study the metabolism and actions of antineoplastic agents under conditions that precisely reflect their clinical pharmacokinetics.

ara-C, which is one of the most active agents in the treatment of acute nonlymphocytic leukemia (3), is rapidly and extensively converted to its inactive form, ara-U, in the human body (4). Daunorubicin is another key drug in leukemic therapy (5). After its administration, daunorubicin rapidly disappears from the plasma by accumulating intracellularly in many tissues (6, 7). Judging from their pharmacokinetic behavior, to evaluate the antineoplastic effects of ara-C and daunorubicin, it is crucial to precisely simulate their plasma pharmacokinetics in clinical use.

Here, we report the cell growth inhibition effects of these drugs on the leukemic cell line K562. The drug concentrations were regulated by a new in vitro pharmacokinetic simulation system that can simulate the changes of drug concentrations in human plasma in vivo using a computer. To the best of the our knowledge, this is the first report of an evaluation of the activities of antineoplastic agents in vitro under simulated conditions that precisely reflect their pharmacokinetics in human plasma.

MATERIALS AND METHODS

Chemicals. ara-C was purchased from Sigma Chemical Co. (St. Louis, MO); Daunorubicin and CDDP (or cisplatin) were kindly supplied from Meiji Seika Co. (Tokyo, Japan) and Nihon Kayaku Co. (Tokyo, Japan), respectively.

Leukemic Cell Lines and Cell Culture. The human chronic myelogenous leukemia cell line K562 was cultured in RPMI 1640 (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 10% FBS (Life Technologies Inc., Grand Island, NY) in a 5% CO₂-humidified atmosphere at 37°C.

In Vitro Pharmacokinetic Simulation System. An in vitro pharmacokinetic simulation system (Fig. 1) that was originally used in the evaluation of activities of an antimicrobial agent (8) was used, with modifications. K562 cells were cultured in the presence of each antineoplastic agent in this system to evaluate the cytotoxic effects of the drugs. Briefly, a controller (ATTO Co., Tokyo, Japan), which was regulated by a software program (Sankyo Automatic Concentration Simulator Version 5.0, Sankyo Co., Tokyo, Japan) on a personal computer (NEC 9801 Vm; Nihon Electronic Co., Tokyo, Japan), inputted the human pharmacokinetic data (two-compartment open model) and regulated the peristaltic pump (Perista Pump; ATTO). Changes in plasma concentration of drugs in human bodies can be precisely simulated in the culture bottle of this system.

The body surface area was set as 1.5 m². The system was set up to simulate the plasma concentration-time curve of clinically intermediate-dose ara-C therapy (1.0 g/m² infusion, for 2 h), using the data that we reported previously (9): Vc (volume of the central compartment), 27.9 liters; Ke (rate constant for elimination from the central compartment), 13.8/h; K0 (rate constant for movement from the peripheral to the central compartment), 4.18/h; α (rate constant for the α or initial phase), 21.3/h; and β (rate constant for the β or second phase), 3.13/h. Regular-dose ara-C therapy (100 mg/m²) as a 2-, 4-, 8-, or 16-h infusion was also simulated so that each AUC was the same. For the 8- and 16-h infusion simulations, the medium was exchanged at 4 and 8 h, respectively, from the beginning of the drug addition to avoid the inactivation of ara-C and daunorubicin, which is another key drug in leukemic therapy (5).

The abbreviations used are: ara-C, 1-β-D-arabinofuranosylcytosine; ara-U, 1-β-D-arabinofuranosyluracil; CDDP, cis-diaminedichloroplatinum; FBS, fetal bovine serum; AUC, area under the serum concentration-time curve; HPLC, high-performance liquid chromatography.

Received 10/26/98; accepted 3/30/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
of ara-C in the long-term cell-suspended culture medium of the simulation system (Fig. 2A). The end point of the medium exchange was set at 0.005 μg/ml to avoid excessive cell reduction by dilution. This concentration was found to have no substantial antineoplastic effect in each drug in this experimental system (data not shown).

The pharmacokinetic data concerning daunorubicin reported previously (10) were reanalyzed using the nonlinear multiple regression computer program MULTI2 (11). Using the data obtained ($V_c$, 79.7 liters; $K_{10}$, 6.31/h; $K_{21}$, 0.92/h; $\alpha$, 17.7/h; and $\beta$, 0.27/h), the simulations of 40, 50, and 60 mg/m² daunorubicin for a 30-min infusion were set up. In addition, 50 mg/m² daunorubicin as a 0.5-, 2-, 4-, 8-, and 16-h infusion was also simulated (Fig. 2B). The end point of the medium exchange in the eliminating phase was set at 0.005 mg/ml daunorubicin, which was also demonstrated to have no substantial antineoplastic effect in this system (data not shown).

The AUCs and $C_{max}$ (maximal concentrations) of each experiment are shown in Table 1. The AUC was calculated from the beginning of the drug addition to the end of the medium exchange.

Conventional Culture System. In the presence of ara-C, daunorubicin, or CDDP, cells were cultured for fixed exposure times, washed twice with RPMI 1640, and resuspended with RPMI 1640 containing 10% FBS. The cell-killing effects of drugs was determined 24 h after the drug addition. The $C \times T$ (concentration-time product) and $C_{max}$ values for ara-C and daunorubicin are shown in Table 1 for comparison with the simulation system.

Evaluation of Cell Killing Effect of Drugs. The cell-killing effects of the drugs were evaluated by a modified semisolid clonogenic culture technique (12). Briefly, the number of viable cells, determined by a trypan blue dye exclusion assay 24 h after the addition of the drug, was adjusted to 400 cells/ml in RPMI 1640 with 1.7% methylcellulose as viscous support and 6% FBS. A 0.5-ml aliquot of this mixture was placed in a 10-mm dish (in triplicate) and incubated at 37°C in humidified air with 5% CO₂ for 6–8 days. After this incubation, the colonies that contained over 30 cells were counted.

Measurement of Drug Concentration. ara-C samples from the culture bottles were collected in to sample tubes containing tetrahydrouridine (final concentration, 0.1 mM) and then centrifuged at 300 × g for 4 min. The supernatants were applied to HPLC. For the HPLC analysis, we used a CCPM 880-PU intelligent pump (Tosoh Corp., Tokyo, Japan), together with an injection valve (model 7125; Rheodyne Co., Catati, CA) equipped with a 100-μl loop UV detector (UV-8000; Tosoh) and a Chromatocorder 21 (Tosoh). Fifty μl of the supernatant were injected onto an ion-exchange column, Partisil 10SCX (Whatman Inc., Clifton, NJ). The elution was performed with 0.01 M ammonium formate (pH 3.0) at a constant flow rate of 1.5 ml/min. The column

Fig. 1. The in vitro pharmacokinetic simulation system. A controller that received signals from the programmed computer regulated the pumps. Pump 1 and pump 2 were for the drug supplements, and pump 3 and pump 4 were for medium exchange in the culture bottle. The stirrer was for mixing the culture medium.

Fig. 2. The time course of drug concentration in the culture system by application of the in vitro pharmacokinetic simulation system. A, simulation of regular-dose (100 mg/m²) ara-C therapy. The simulations of a 2-h infusion (---), and 16-h infusion (----) are shown. In the simulation of the 16-h infusion, the ara-C-containing medium was exchanged at 8 h (arrow) from the beginning of drug addition to avoid the effect of ara-C inactivation in the long-term cell-suspended culture medium. B, simulation of daunorubicin (50 mg/m²) infusions for 30 min (---), and for 4 h and 8 h (----). The end point of the medium exchange was 0.005 μg/ml at its eliminating phase in both simulations. The practically measured daunorubicin concentration detected by a spectrophotometer is plotted (○). The concentrations of measured daunorubicin at 2, 4, 6, and 24 h were 0.021, 0.029, 0.008, and 0.0065 μg/ml, respectively (the expected values were 0.023, 0.029, 0.009, and 0.005 μg/ml, respectively).
eluate was monitored at 280 nm, which corresponds to the wavelength of the maximum UV absorption of ara-C. The retention time was 15 min, and the limit of detection was 0.01 μg/ml.

For the determination of ara-C production using [3H]ara-C, an aliquot of supernatant was spotted on a thin-layer silica gel sheet (Polygram CEL 300 UV254; Machert-Nagel Co., Du¨ren, Germany). The sample was developed with chloroform:methanol:acetic acid (20:5:1) for 2 h. Daunorubicin samples from culture bottles were collected in sample tubes and then centrifuged at 300 × g for 4 min. The daunorubicin concentration of each supernatant was determined by a spectrofluorophotometer (model RF-5000; Shimadzu Corp., Kyoto, Japan); the excitation and emission wavelengths were 467 and 600 nm, respectively. The stability of [3H]daunorubicin during the incubation was determined by thin-layer silica gel sheets (TLC plastic sheets UV254; Machert-Nagel Co., Du¨ren, Germany). The sample was developed with 2-propanol:methylacetate:water (2:2:1) for 3 h. The conversion ratio of ara-C to ara-U was calculated as the radioactivity of ara-U aliquot divided by total radioactivity on the sheet.

Daunorubicin samples from culture bottles were collected in sample tubes and then centrifuged at 300 × g for 4 min. The daunorubicin concentration of each supernatant was determined by a spectrofluorophotometer (model RF-5000; Shimadzu Corp., Kyoto, Japan); the excitation and emission wavelengths were 467 and 600 nm, respectively. The stability of [3H]daunorubicin during the incubation was determined by thin-layer silica gel sheets (TLC plastic sheets UV254; Machert-Nagel Co., Du¨ren, Germany). The sample was developed with chloroform:methanol:acetic acid (20:5:1) for 2 h. Daunorubicin and its metabolites (daunorubicinol and the aglycones) were separated as described elsewhere (13). The stability of daunorubicin was calculated by the radioactivity of daunorubicin after incubation for a designated time divided by the radioactivity at the start of incubation, using a liquid scintillation counter (model LSC-3500; Aloka Corp., Tokyo, Japan). The radioactivity of an aliquot of supernatant was also determined by a liquid scintillation counter.

Statistical Analysis. The statistical analysis was carried out using a computer program, StatView (Abacus Concepts, Inc., Berkeley, CA). Differences of P < 0.05 were considered significant.

### Table 1

<table>
<thead>
<tr>
<th>Simulation system</th>
<th>Conventional culture system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (h)</td>
<td>AUC (μm · h)</td>
</tr>
<tr>
<td>Ara-C 2</td>
<td>1.59</td>
</tr>
<tr>
<td>4</td>
<td>1.59</td>
</tr>
<tr>
<td>8</td>
<td>1.60</td>
</tr>
<tr>
<td>16</td>
<td>1.60</td>
</tr>
<tr>
<td>Daunorubicin 0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>2</td>
<td>0.25</td>
</tr>
<tr>
<td>4</td>
<td>0.25</td>
</tr>
<tr>
<td>8</td>
<td>0.25</td>
</tr>
<tr>
<td>16</td>
<td>0.25</td>
</tr>
</tbody>
</table>

* CxT, concentration-time product; Cmax, maximal concentration.

Fig. 3. Cell-killing effect of ara-C on K562 cells. A, application of the in vitro pharmacokinetic simulation system. ■ simulation of intermediate-dose ara-C therapy (1.0 g/m² for a 2-h infusion); □ simulation of regular-dose ara-C therapy (100 mg/m² for 2-, 4-, 8-, and 16-h infusions). The number of K562 cells was adjusted to 2.5 × 10⁶/ml at 24 h before each experiment in RPMI 1640 containing 10% FBS. B, application of conventional culture system. The ara-C concentrations were 0.80, 0.40, 0.20, and 0.10 μM at the exposure times of 2, 4, 8, and 16 h, respectively. The number of K562 cells was adjusted to 2.5 × 10⁶ cells/ml at 24 h before each experiment in RPMI 1640 containing 10% FBS. The relative cell survival in each experiment was determined by semisolid clonogenic culture technique as described in “Materials and Methods.” Columns, means of at least three determinations; bars, SD. * Mann-Whitney’s U test; **, one-way factorial ANOVA was applied, and Fisher’s PLSD test as post hoc test revealed significant differences between 2- and 8-h, 2- and 16-h, and 4- and 16-h infusion times.

### RESULTS

#### Accuracy of the in Vitro Pharmacokinetic Simulation System.

To test the accuracy of the in vitro pharmacokinetic simulation system, we measured the ara-C concentration at 4, 29, 119, 127, 179, and 254 min after the start of the simulation of intermediate-dose ara-C therapy by HPLC in the cell-free condition. The ara-C concentrations were 0.87, 1.81, 2.20, 0.74, and 0.04 μg/ml and under the limit of detection, respectively. The discrepancy between these values and the expected values (1.02, 1.77, 1.94, 0.73, 0.04, and 0.005 μg/ml, respectively) was 6.1 ± 7.3% (mean ± SD). For daunorubicin, the discrepancy against the expected values was 10.1 ± 8.4% (2, 4, 6, and 24 h after the start of drug addition) in the 50 mg/m² daunorubicin infusion simulation for 4 h (Fig. 2B). The in vitro pharmacokinetic simulation system was concluded to be adequately accurate in light of these data.

#### Cell-killing Effect of ara-C in the in Vitro Pharmacokinetic Simulation.

For the intermediate dose (1.0 g/m² for the 2-h infusion), the relative survival rate of K562 cells in the presence of ara-C compared with the control (no drug addition) was 7.1% under the conditions that simulated intermediate-dose ara-C therapy by the application of an in vitro pharmacokinetic simulation system (Fig. 3A).

With the simulated regular-dose (100 mg/m² for 2-, 4-, 8-, and 16-h infusions) ara-C therapy, in which the ara-C was kept constant, the relative survival rates following the 2-, 4-, 8-, and 16-h infusions were 75, 72, 34, and 14% using clonogenic culture technique, respectively, compared with the control (Fig. 3A). Definite time-dependent inhibition by ara-C was observed.

#### Cell-killing Effect of ara-C in the Conventional Culture System.

The cell-killing effect of ara-C measured by the conventional culture system is shown in Fig. 3B. When the C × T values of ara-C were made constant, the relative cell survival values compared with the control were 51, 60, 60, and 65% for ara-C exposure times of 2, 4, 8, and 16 h and ara-C concentrations of 0.8, 0.4, 0.2, and 0.1 μM, respectively. In this conventional culture system, no time dependency of ara-C was demonstrated.

#### Inactivation of ara-C in the Conventional Culture System.

When K562 cells (adjusted to 2.5 × 10⁶ cells/ml at 24 h before each experiment at the beginning in both systems) were incubated in the presence of 0.2 μM [3H]ara-C, the conversion rate of ara-C to ara-U in the cell-suspended medium in the conventional culture system was...
The system was set up so that 40, 50, or 60 mg/m² of daunorubicin was administered as a 30-min infusion, the relative cell survival rates were 76.0, 36.3, and 16.3%, respectively, compared with the control (Fig. 5A). In the simulation of 50 mg/m² daunorubicin administered as 0.5-, 2-, 4-, 8-, and 16-h infusions, the relative survival values were 37.4, 49.7, 72.1, 82.2, and 87.4%, respectively, compared with the control (Fig. 5B).

Cell-killing Effects of Daunorubicin in the in Vitro Pharmacokinetic Simulation. When the in vitro pharmacokinetic simulation system was set up so that 40, 50, or 60 mg/m² of daunorubicin was administered as a 30-min infusion, the relative cell survival rates were 76.0, 36.3, and 16.3%, respectively, compared with the control (Fig. 5A). In the simulation of 50 mg/m² daunorubicin administered as 0.5-, 2-, 4-, 8-, and 16-h infusions, the relative survival values were 37.4, 49.7, 72.1, 82.2, and 87.4%, respectively, compared with the control (Fig. 5B).

Cell-killing Effects of Daunorubicin and CDDP in the Conventional Culture System. When the daunorubicin exposure times were 0.5, 2, 4, 8, and 24 h and the daunorubicin concentrations were 0.48, 0.12, 0.06, 0.03, and 0.01 μM, respectively, with a constant daunorubicin C × T, the relative survival values compared with the control were 7.6, 18.6, 63.7, 92.0, and 96.3% (Fig. 6A).

To examine whether daunorubicin is AUC-dependent, the IC₅₀ (drug concentration for 90% cell kill) versus exposure time plot on a double-logarithmic scale of daunorubicin was compared with that of CDDP, which is well known to be AUC dependent (14), in the conventional culture system. The regression plot of the daunorubicin had the slope −0.40 (Fig. 6B), whereas the regression line of CDDP was at −0.98 (Fig. 6C).

Stability of Daunorubicin and Change of Daunorubicin Concentration in the Cell-suspended Medium. After the incubation of K562 cells with 0.1 μM of [³H]daunorubicin for 4 and 24 h in the conventional culture system, 96.3 ± 15.2% and 89.8 ± 16.0% (mean ± SD of triplicate experiments), respectively, of total radioactivities remained in the cell-suspended medium. The residual rates of unmetabolized daunorubicin in the supernatant were 92.3 ± 4.9% and 93.4 ± 3.2% (mean ± SD of quadruplicate experiments), respectively, compared with 0 h of incubation, suggesting that daunorubicin was substantially stable during the incubation for ~24 h. The number of K562 cells was adjusted to 2.5 × 10⁷ cells/ml at 24 h before each experiment in RPMI 1640 containing 10% FBS.

DISCUSSION

An analysis of leukemic cells obtained directly from a patient immediately after drug administration would be the most accurate method for evaluating how an antileukemic agent is metabolized and acts in tumor cells when it is clinically administered. However, it is rare that an adequately large amount of cells is obtained from a leukemia patient. Thus, for evaluating the metabolism and action of drugs, the application of an in vitro pharmacokinetic simulation system is very useful because of its reproducibility and ease of use.

After intracelluar conversion to the corresponding nucleotide, ara-C acts as an antagonist of its physiological substrate, dCTP, and competitively inhibits DNA polymerase (15). ara-C triphosphate is also incorporated to a limited extent into DNA (16), ara-C is classified as a cell cycle phase-specific agent (17) or time-dependent drug (14). We observed a clear time dependency of the cytotoxicity of ara-C using the in vitro pharmacokinetic simulation system (Fig. 3A), whereas this time dependency was not shown using the conventional culture system model (Fig. 3B). This difference was probably due to the fact that ara-C was rapidly converted to ara-U in the cell-suspended culture medium in the conventional culture system (Fig. 4). In the in vitro pharmacokinetic simulation, the number of cells decreased as the drug concentration changed at its eliminating phase because the concentration was adjusted by mixing drug-containing and drug-free media, regulated by the computer. In addition, to avoid slow but

Fig. 4. Conversion rate of ara-C to ara-U in a 24-h incubation in the conventional culture system. K562 cells, adjusted to 2.5 × 10⁵ cells/ml at 24 h before each experiment, were incubated in the presence of 0.2 μM [³H]ara-C in the conventional culture system. Data points, means of two determinations. The conversion rate of ara-C was measured as described in “Materials and Methods.”

Fig. 5. Relative survival rates of K562 cells in simulated daunorubicin infusions. A, with a fixed infusion time (30 min), the daunorubicin dose was altered. The number of K562 cells was adjusted to 2.5 × 10⁵ cells/ml at 24 h before each experiment in RPMI 1640 containing 10% FBS. B, with a fixed daunorubicin dose (50 mg/m²), the infusion time was altered. The number of K562 cells was adjusted to 1.5 × 10⁵–2.5 × 10⁵ cells/ml at 24 h before each experiment in RPMI 1640 containing 10% FBS. Columns, means of at least three determinations; bars, SD. One-way factorial ANOVA was applied, and Fisher’s PLSD test as post hoc test revealed significant differences between 40 and 50 mg/m² and between 40 and 60 mg/m² (A) and between the 0.5- and 4-h, 0.5- and 8-h, 0.5- and 16-h, 2- and 8-h, and 2 and 16-h infusions (B).

p < 0.01

p = 0.005

A

B

2632
persistent death by residual cells in the case of long-term exposure to ara-C, the medium in the culture bottle was exchanged 4 and 8 h after the drug addition in the simulations of 8- and 16-h infusions, respectively (Fig. 2A). By this medium exchange, the ara-C concentration was essentially maintained the simulated curve (data not shown).

An important pharmacological characteristic of daunorubicin is DNA intercalation. Daunorubicin inserts itself into the DNA double helix in such a fashion that the aglycone moiety is between the adjacent bp and parallel to them (18). Once binding to DNA occurs, several events may ensue: blockage of the synthesis of DNA, RNA, and protein; fragmentation of the DNA; and inhibition of DNA repair have all been reported (19), although the main mechanism of action is thought to be the inhibition of topoisomerase-2 (20, 21). Daunorubicin is classified as a non-cell cycle phase-specific agent (17).

Ozawa et al. (14, 22) demonstrated that the cell-killing action of non-cell cycle-specific agents such as CDDP or nitrogen mustard depends on $C \times T$ or AUC. They also demonstrated that when the $IC_{50}$ and exposure times were plotted on a logarithmic-scale graph, if the cytotoxicity of the drug depends on the $C \times T$ or AUC, a linear relationship with the slope of $-1$ is seen (22). Our present results are in agreement concerning CDDP but not daunorubicin, as shown in Fig. 6, B and C. The regression plot of daunorubicin had the slope $-0.40$, indicating that the same $C \times T$ does not produce the same cell growth-inhibitory effect at various daunorubicin exposure times. We examined whether daunorubicin was metabolized like ara-C. Daunorubicin was proven to be substantially stable during the incubation; i.e., for the same cell-killing effects ($IC_{50}$), higher $C \times T$ values of daunorubicin are needed at long-term exposures compared to short-term exposures. We also found that, when the $C \times T$ value of daunorubicin in each incubation time, was kept constant, the cell growth inhibition effect was reduced as the exposure time was prolonged (Fig. 6A). These results suggest that the cell-killing action of daunorubicin depends on the $C_{\text{max}}$ rather than the $C \times T$. Previous studies also showed that with a similar intracellular exposure dose, a greater cytotoxicity effect was achieved after incubation with a high drug concentration of daunorubicin or doxorubicin for a short time compared to after incubation with a lower concentration for a longer time (23, 24). Greater $C_{\text{max}}$ dependence would be expected with a very active export pump. However, we previously reported the characteristics of the K562 cell line used for our experiments as wild type; it has no multidrug resistance activity (25).

In the clinical use of anthracyclines, including daunorubicin, it was reported that long-term infusions of these drugs reduce their toxic effects, especially cardiotoxicity (26), whereas the antineoplastic effect is the same as that in a rapid infusion (6, 27, 28). Hortobagyi et al. (29) reported that long infusions of doxorubicin in combination chemotherapy produce similar effects to shorter infusions or boluses while partly protecting the heart. They showed that there was no difference in survival duration between the two groups, although the cumulative doses of doxorubicin were higher in the continuous-infusion group because of its reduced cardiotoxicity. The vincristine-doxorubicin by continuous infusion with intermittent dexamethasone regimen, given by continuous infusion along with a frequent administration of dexamethasone, has achieved a marked tumor reduction in about half of the patients (30). There are some reports that dexamethasone accounted for most of the plasma cell reduction achieved by vincristine-doxorubicin by continuous infusion with intermittent dexamethasone therapy in previously untreated or unresponsive patients with multiple myeloma (31, 32). Another study concerning cellular pharmacokinetics of doxorubicin in patients with chronic lymphocytic leukemia demonstrated that the cellular AUC of doxorubicin after a bolus injection was 2.85 times greater than that observed after a continuous administration (33). A continuous infusion of anthracycline might not be as efficacious as bolus infusion against tumor cells.

We established an in vitro pharmacokinetic simulation system in which the concentration of daunorubicin in the medium simulated that administered i.v. When the AUC kept constant for each infusion time, the cell-killing effect was reduced as the infusion time was prolonged (Fig. 5B), as was also observed in the conventional culture system. However, this decrease of cytotoxicity was less in the in vitro simulation than in the conventional culture. The difference between the two systems may be caused by a smaller $C_{\text{max}}$ in the simulation system.
compared with the conventional system under the conditions of the same AUC and incubation time because of the former system’s relatively long $\beta$ phase (Table 1). The decrease of cytotoxicity based on the lesser $C_{\text{max}}$ fluctuation in the in vitro pharmacokinetic simulation may more accurately simulate the clinical finding that the antineoplastic effect of a long-term daunorubicin infusion seems to be essentially the same as that of a short-term infusion, compared to the conventional culture system. Prospective clinical and pharmacokinetic studies with large numbers of cases are needed to determine whether a continuous infusion and shorter-term infusion have similar effects regarding the tumor reduction.

Comparing the cytotoxicity of daunorubicin with that of ara-C, when the infusion time was kept constant, a smaller change of the daunorubicin dose resulted in a greater change in the survival rate compared to ara-C. For example, an only 1.5-fold increase in the dose of daunorubicin resulted in a 5-fold increase in cytotoxicity, whereas a 10-fold increase in the dose of ara-C resulted in a 10-fold increase in cytotoxicity, as shown in Figs. 5A and 3A. We suspect that this is due to the difference in the cell-killing action of the two drugs. The cell-killing effect of daunorubicin is $C_{\text{max}}$ dependent and that of ara-C is time dependent. In the clinical use of $C_{\text{max}}$-dependent antineoplastic agents, such as daunorubicin, careful management of the dose is needed, because a small change in the infusion dose results in a large change in the therapeutic effect. In contrast, careful management of the setting time is needed when using time-dependent drugs such as ara-C.

A limitation of our system might involve compounds that undergo species-specific protein binding, such as human albumin (3A, 35), because the FBS was used in the system. We confirmed that daunorubicin was substantially stable in the culture medium. It may be possible to overcome this limitation using tissue culture medium fortified with human plasma proteins. Furthermore, this technology might be useful using other agents that are unstable in human plasma, such as topotecan (36).

In conclusion, it is difficult to predict a drug’s precise cell growth-inhibitory effect in the clinical use of antineoplastic agents, especially antimitobolites, using only a conventional culture system. This simulation system can predict the effects of ara-C, daunorubicin, and other antineoplastic agents much more exactly compared to the conventional culture system in clinical use. We have confirmed the usefulness of the evaluation of antineoplastic agents such as ara-C and daunorubicin by the application of our new in vitro pharmacokinetic simulation system.

ACKNOWLEDGMENTS

We thank Dr. S. Oya of Sankyo Co. Ltd. (Tokyo, Japan) for his valuable suggestions and assistance with the in vitro pharmacokinetic simulation model, Dr. Y. Urasaki for his valuable advice regarding the use of the computer, Dr. T. Yamashita for his constructive advice regarding ara-C, and Drs. T. Fukushima and H. Takemura for advice concerning daunorubicin. We also thank T. Nakamichi for her excellent secretarial and technical assistance.

REFERENCES

Evaluation of Cell-killing Effects of 1-β-d-Arabinofuranosylcytosine and Daunorubicin by a New Computer-controlled in Vitro Pharmacokinetic Simulation System


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/59/11/2629

Cited articles
This article cites 34 articles, 7 of which you can access for free at:
http://cancerres.aacrjournals.org/content/59/11/2629.full#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/59/11/2629.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.