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Paclitaxel effect on a leukemia cell line: importance of paclitaxel concentration

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The efficacy of a drug may be a complex function of the length of drug exposure and the drug concentration. One traditional way to model these variables together has been to look at the area under the curve (AUC) when drug concentration is plotted against time. Experiments in an HL60 leukemia cell model suggest that, for the antineoplastic drug paclitaxel, AUC is not a good predictor of efficacy.

Key words: Leukemia, paclitaxel, pharmacokinetics, taxol.

Introduction

Paclitaxel ($C_{47}H_{51}N_{14}$, molecular weight 853.9) is now used widely in clinical oncology. Much of the initial experience has been with the treatment of ovarian carcinoma on a 24 h infusion schedule. Subsequently there has been considerable interest in the use of paclitaxel (taxol) for other neoplastic diseases, including breast cancer and lymphoma.

A 3 h paclitaxel infusion schedule is more convenient for patients than the original 24 h schedule and has recently been added to the American package label indications for this agent. The implicit basis for this change has been that the patient can get a comparable clinical effect with a short infusion schedule of paclitaxel that delivers a similar area under the curve (AUC) of paclitaxel concentration multiplied by time, even though the short infusion schedule produces an approximately 10-fold higher peak paclitaxel concentration.

In the clinical setting there is only a limited ability to manipulate peak paclitaxel concentration as a viable separate from AUC. Paclitaxel is administered as an infusion of 3 h or more under the protocols in common clinical use. Once a plasma peak is attained in the patient, it is cleared in a rather leisurely fashion. The manufacturer claims a $T_{1/2}$ of 13.1 h (for a 3 h infusion at 135 mg/m^2) or 20.2 h (for a 3 h infusion at 175 mg/m^2).[1] A recent published report describes a triphasic model for paclitaxel clearance at these dose levels, likewise with a prolonged terminal phase (respectively 14.4 and 18.8 h).[2] As a result, in the clinical setting a high peak paclitaxel concentration is necessarily associated with a substantial paclitaxel AUC.

It is difficult *in vivo* to study peak concentration separately from AUC, but the distinction may well be important for understanding the toxicity of paclitaxel. Recent data suggest that paclitaxel AUC is a good predictor of the neuromuscular toxicity of paclitaxel, but not of paclitaxel's hematologic toxicity.[3] The present studies used an *in vitro* system to study the effect of the schedule of paclitaxel administration on its toxicity for a myeloid leukemia cell line.

Materials and methods

Cells of the HL60 cell line (American Type Culture Collection, Rockville, MD) were grown in a mixture of 79% RPMI 1640 medium with HEPES and L-glutamine (Gibco, Grand Island, NY) supplemented with 20% heat-inactivated fetal calf serum (Sigma, St Louis, MO) and 1% penicillin/streptomycin (Gibco), in a 37°C, 5% CO_2 humidified incubator, and carried in culture by serial passage. Cells were counted by hemocytometer, with viability evaluated by Trypan blue exclusion, in order to calculate the viable cell counts. Experiments were routinely set up with three sequential sets of triplicate samples (with each at an initial cell count of 50 viable cells/mm^3), so that each data point is based on the mean of nine samples. Cultures were inspected grossly and microscopically for signs of contamination during the course of the experiments.

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Data analysis

Data analysis was based on the viable cell counts 1 week after subculturing, because this initial week is a time period where (as shown in previous experimentation from this laboratory) one would expect exponential growth in the absence of chemotherapy. The yield of viable HL60 cells was determined under control (no paclitaxel) or under a variety of drug exposure conditions. Analysis of variance was computed for each set of experimental data, using the viable cell counts at day seven. The α value for the F test was set at 0.01 to test the null hypothesis that there was no significant difference produced by any of the choices of chemotherapy exposure. If this preliminary testing refuted the null hypothesis, then Tukey's HSD (honestly significant difference) test was used to determine, at an α level of 0.01, which pairs of experimental conditions were associated with significantly different viable cell counts on day 7.[4]

The experimental cultures were exposed for the selected period of drug treatment to paclitaxel obtained from a commercial chemical supplier (taxol; Calbiochem San Diego, CA). Within each experiment reported below, drug exposure was done beginning at time 0. In some cases cells remained in paclitaxel-containing media for an entire week, while in other cases the cells were spun down and resuspended in drug-free media after an initial period of exposure to paclitaxel. Viable cell counts were determined at 1 week after the start of the drug exposure period. Paclitaxel AUC was calculated based on the product of the initial paclitaxel concentration multiplied by the amount of time of the paclitaxel exposure.

Results

An initial series of experiments were performed to identify the level of paclitaxel that was moderately toxic to HL60 cells. Paclitaxel concentrations of 0.01 μg/ml (0.012 μM) or less produced retardation of cell growth in some experiments, but there was some variation in the sensitivity to these very low concentrations of paclitaxel. Initial experiments suggested that with prolonged exposure to paclitaxel, doses as low as 0.001 μg/ml could produce some toxicity. In another experiment, paclitaxel 0.1 μg/ml was markedly toxic for HL60 cells even on a 1 h exposure.

Subsequent experiments were performed to compare the growth of HL60 cells under drug-free conditions to HL60 cells exposed to a particular paclitaxel AUC (the product of paclitaxel concentration multiplied by the length of the drug exposure period). AUC within a given experiment was identical for all the drug-exposed cells, but some cells had a brief exposure to a relatively high concentration of paclitaxel, while other cells had a longer exposure to a correspondingly lower paclitaxel concentration.

Cells in one such experiment were grown either drug-free (Figure 1E) or else exposed to a paclitaxel AUC of 0.05 μg h/ml (Figure 1A–D). The drug exposures used to generate this AUC ranged in length from 1 h (at 0.05 μg/ml) to 25 h (at 0.002 μg/ml). Cell growth occurred in all five conditions at this AUC, so that in each case the mean viable cell count at day 7 exceeded the initial viable cell count of 50 cells/mm^3.

Subsequent series of experiments used the same range of time for drug exposure but at higher paclitaxel concentrations (producing correspondingly higher AUC levels). At an AUC of 0.1 μg h/ml, a 1 h exposure at 0.1 μg/ml was the most toxic choice. (Figure 2A). An AUC of 0.5 μg h/ml showed marked toxicity at a variety of dose schedules, but here again brief intense courses of paclitaxel were more potent than prolonged (25 h, 0.02 μg/ml) paclitaxel therapy at the chosen AUC (Figure 3).

The effect of paclitaxel concentrations below 0.1 μg/ml were weak and inconsistent. The series of experiments at an AUC of 0.05 μg h/ml suggest that paclitaxel concentrations as low as 0.002 μg/ml could slow the rate of HL60 cell growth compared with control cells (Figure 1), but a cytostatic effect for low concentration paclitaxel was not apparent in the series of experiments at 0.1 μg h/ml (Figure 2).

The consistent finding among the various experiments was that a paclitaxel concentration of at least 0.1 μg/ml, even if administered for as short a period as 1 h, was markedly cytotoxic for HL60 cells. Even a brief exposure to paclitaxel above this threshold concentration caused the mean viable cell count at day seven to be less than the initial value of 50 cells/mm^3.

Discussion

The present experiments used *in vitro* pulse exposures to paclitaxel, generally followed by a resuspension in paclitaxel-free media. The design of the present experiments used a constant dose level for a set period of time, so that peak and trough concentrations were presumed to be identical. (Actual pa-

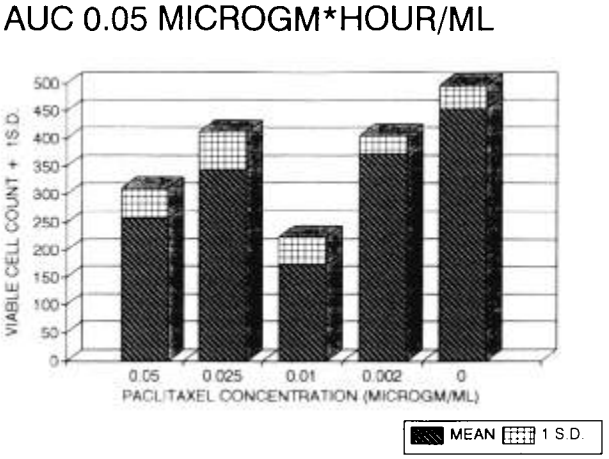

Figure 1.

	Paclitaxel (μg/ml)	Hours of exposure
A	0.05	1
B	0.025	2
C	0.01	5
D	0.002	25
E	0	0

Cells were exposed to either no paclitaxel (control condition E) or to an AUC of 0.05 μg h/ml of paclitaxel. The graph shows the mean (of nine separate samples for each experimental condition) viable cell count and standard deviation a week after the start of the exposure. The *F* test (with a variance ratio of 35.6) demonstrated that the five experimental conditions were non-identical in their results and the HSD test results below show which of the conditions differed on pairwise comparison. The results indicated by an asterisk are those where the absolute difference in viable cell count per mm^3 on pairwise comparison was 74 or greater, significant at the *p* 0.01 level:

	A	B	C	D	E
A	—	*89	*84	*116	*197
B	*89	—	*173	27	*108
C	*84	*173	—	*200	*281
D	*116	27	*200	—	*81
E	*197	*108	*281	*81	—

clitaxel concentrations were not assayed during the course of the experimental exposure, so it is possible that the paclitaxel concentration in the medium could have fallen somewhat during the course of a 24 h exposure, due to paclitaxel degradation and/or to paclitaxel uptake from the medium into the HL60 cells.) The resuspension in drug-free media causes the paclitaxel level to be brought down rapidly at the end of a pulse exposure, rather than falling slowly as it would *in vivo*. The data demonstrate that AUC for paclitaxel in some circumstances is not the best predictor of paclitaxel toxicity for HL60 cells, but rather that paclitaxel concentration is sometimes more important for toxicity.

In the clinical setting, the patient's paclitaxel level will fall gradually at the end of a paclitaxel infusion, over a period of many hours. Recent evidence suggests that the degree of hematologic toxicity reflects the length of time that the patient's paclitaxel level remains above a threshold value of 0.1 μM. Clinical trials have documented hematologic toxicity as an important factor in limiting patient tolerance of paclitaxel, and granulocyte colony stimulating factor has been used to ameliorate paclitaxel-induced neutropenia.[5] If the effect on a patient's tumor is influenced heavily by the paclitaxel concentration, while the hematologic toxicity reflects the length of time of the paclitaxel exposure, then the best therapeutic index is likely to be achieved by a very brief exposure to high-dose paclitaxel.

The data obtained here demonstrating the importance of paclitaxel concentration were in HL60, a myeloid leukemia cell line. HL60 proved quite sensitive to paclitaxel at concentrations well below the

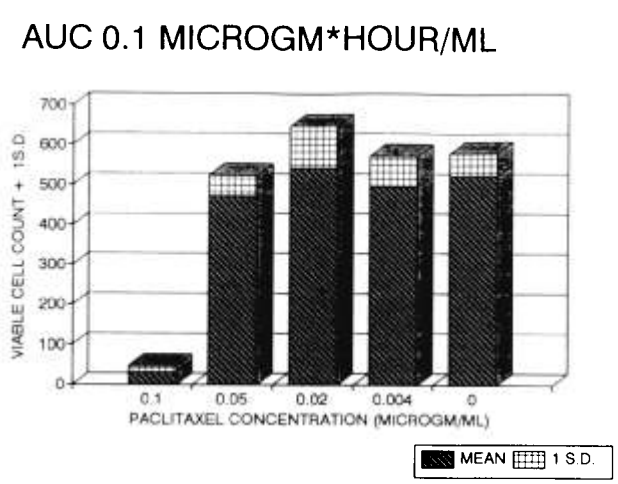

Figure 2.

	Paclitaxel (μg/ml)	Hours of exposure
A	0.1	1
B	0.05	2
C	0.02	5
D	0.004	25
E	0	0

Cells were exposed to either no paclitaxel (control condition E) or to an AUC of 0.01 μg h/ml of paclitaxel. The graph shows the mean (of nine separate samples for each experimental condition) viable cell count and standard deviation a week after the start of the exposure. The *F* test (with a variance ratio of 78.9) demonstrated that the five experimental conditions were non-identical in their results and the HSD test results below show which of the conditions differed on pairwise comparison. The results indicated by an asterisk are those where the absolute difference in viable cell count per mm^3 on pairwise comparison was 98 or greater, significant at the *p* 0.01 level:

	A	B	C	D	E
A	—	*441	*512	*467	*493
B	*441	—	72	26	52
C	*512	72	—	45	19
D	*467	26	45	—	26
E	*493	52	19	26	—

peak concentrations commonly achieved in clinical practice. It is uncertain whether similar results would be obtained with cells of other hematoloic malignancies, or with solid tumors, but this same methodology could readily be used to evaluate such tumor samples. In particular, this method allows one to see whether the given cells are more sensitive to paclitaxel AUC, the length of time of exposure to paclitaxel or to paclitaxel concentration.

A point of special interest would be to identify a difference in toxicity between normal hematopoietic cells and a patient's tumor cells, so that one can improve the therapeutic index of cancer therapy. This strategy could be employed, for example, in the case of a woman with metastatic breast cancer being considered for autologous bone marrow transplantation, a situation where occult contamination of the marrow with metastatic breast cancer is likely.[6] Autologous bone marrow could readily be exposed *ex vivo* to a pulse of high-dose paclitaxel as a purging agent, to produce a high peak paclitaxel level with only a modest paclitaxel AUC. Paclitaxel dose escalation in the setting of bone marrow purging *ex vivo* is particularly attractive because the only dose-limiting toxicity of concern will be that for the normal hematopoietic precursors, and the patient would not be exposed to the risk of neuromuscular toxicity.[7,8] In a similar fashion, one could use intraperitoneal paclitaxel for the therapy of ovarian cancer patients with malignant ascites, in the hope of attaining a high peak intraperitoneal concentration with a low AUC.[9]

Paclitaxel therapy can fail because of the emergence of paclitaxel resistance during therapy.[10] A paclitaxel treatment administered as a brief but intense exposure might prove a useful strategy to simultaneously minimize not only the selection for paclitaxel resistance by tumor cells, but also the

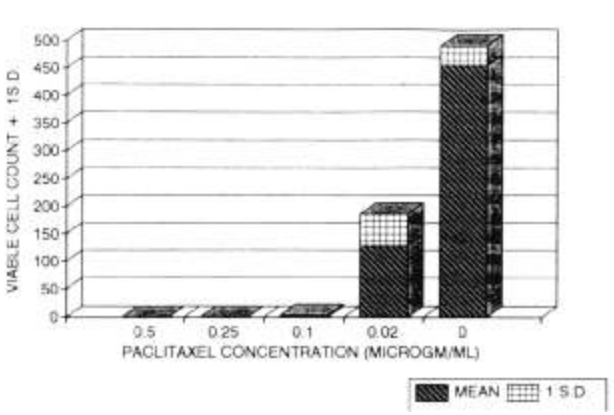

Figure 3.

	Paclitaxel (μg/ml)	Hours of exposure
A	0.5	1
B	0.25	2
C	0.1	5
D	0.02	25
E	0	0

Cells were exposed to either no paclitaxel (control condition E) or to an AUC of 0.5 μg h/ml of paclitaxel. The graph shows the mean (of nine separate samples for each experimental condition) viable cell count and standard deviation a week after the start of the exposure. The *F* test (with a variance ratio of 336) demonstrated that the five experimental conditions were non-identical in their results and the HSD test results below show which of the conditions differed on pairwise comparison. The results indicated by an asterisk are those where the absolute difference in viable cell count per mm^3 on pairwise comparison was 44 or greater, significant at the *p* 0.01 level:

	A	B	C	D	E
A	—	0	2	*129	*456
B	0	—	2	*129	*456
C	2	2	—	*127	*454
D	*129	*129	*127	—	*326
E	*456	*456	*454	*326	—

hematologic toxicity of the therapy. Careful consideration of both paclitaxel concentration and paclitaxel AUC appear important in designing new therapeutic strategies.

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