Cremophor Pharmacokinetics in Patients Receiving 3-, 6-, and 24-Hour Infusions of Paclitaxel

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Background: Paclitaxel (Taxol) is a new drug with efficacy against a variety of malignant tumors. The clinical formulation of paclitaxel contains 50% Cremophor EL, a polyethoxylated castor oil vehicle (carrier) that can reverse multidrug resistance (MDR) mediated by P-glycoprotein. Three-hour intravenous infusions of paclitaxel can yield end-of-infusion plasma Cremophor concentrations of 1 µL/mL or more, which are sufficient to reverse MDR in vitro by at least 50%. Despite extensive clinical use, the pharmacokinetics of Cremophor have not been described. Purpose: We studied the pharmacokinetics of Cremophor in patients with ovarian cancer who were undergoing treatment with paclitaxel to determine whether plasma Cremophor concentrations achieved during and following 3-, 6-, and 24-hour drug infusions were similar to those shown to modulate MDR in vitro. Methods: Eleven patients with previously treated (i.e., with platinum-containing chemotherapy regimens) ovarian cancer who were undergoing treatment with paclitaxel to determine whether plasma Cremophor concentrations achieved during and following 3-, 6-, and 24-hour drug infusions were similar to those shown to modulate MDR in vitro. Results: Ten patients were treated with paclitaxel at a dose level of 175 mg/m², and one patient was treated at a dose level of 135 mg/m². At the 175-mg/m² dose level, peak plasma Cremophor concentrations of 1 µL/mL or more were achieved in eight of 10 patients during both the 3-hour and the 6-hour infusions; with the 24-hour infusion, only one patient achieved a peak plasma Cremophor concentration of 1 µL/mL or more. The eight patients who achieved plasma Cremophor concentrations of 1 µL/mL during the 3-hour infusion were above this level 30 minutes into the infusion; the total time that the plasma concentration was greater than 1 µL/mL was 8.9 ± 5.0 hours (mean ± standard deviation; range, 4.1-15.6 hours). For the eight patients who achieved plasma Cremophor concentrations of 1 µL/mL during the 6-hour infusion, the total time that the concentration was greater than 1 µL/mL was 10.2 ± 9.0 hours (range, 0.3-21.9 hours). The patient who received paclitaxel at a dose of 135 mg/m² achieved a peak plasma Cremophor concentration of 1 µL/mL or more only during the 3-hour infusion. Conclusions: Paclitaxel infusions of 3 and 6 hours can result in sustained plasma Cremophor concentrations sufficient for substantial reversal of P-glycoprotein-mediated MDR in vitro. These plasma Cremophor concentrations are not achieved during 24-hour infusions of paclitaxel. [J Natl Cancer Inst 1996;88:1297-1301]

Paclitaxel (Taxol) is a new drug with significant activity against a number of different tumors, including breast, ovarian, non-small-cell lung, and head and neck cancers (1). The infusion duration of paclitaxel has varied from 1 to 96 hours in clinical trials (2-4). The optimal schedule has not been established, but 3- and 24-hour infusions are most widely used. Two mechanisms of paclitaxel resistance have been identified in vitro. One is associated with overexpression of P-glycoprotein (Pgp), which confers multidrug resistance (MDR) (5), and the other involves alterations in tubulin (6). [Paclitaxel stabilizes microtubules, thus disrupting vital cellular processes and cell division (1).] A number of compounds that are capable of reversing Pgp-associated MDR, such as verapamil and cyclosporin, have been identified, and early clinical trials with these modulators have demonstrated responses in patients who were previously refractory to chemotherapy (7,8). Cremophor EL is a polyethoxylated castor oil vehicle that has been shown to reverse Pgp-associated MDR in vitro and in vivo (9,10). This finding is of particular interest because the clinical formulation of paclitaxel contains 50% Cremophor. We have previously demonstrated that 3-hour infusions of paclitaxel result in plasma Cremophor concentrations at the end of infusion of 1 µL/mL or more and that these concentrations are sufficient to reverse MDR in vitro by at least 50% (11). However, our previous study did not measure Cremophor concentrations during the 3-hour infusion period or during more prolonged periods of infusion. The time required to achieve plasma Cremophor concentrations sufficient for substantial reversal of MDR and the duration

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of these plasma concentrations during paclitaxel chemotherapy are likely to be important factors in the potential modulation of MDR.

Despite extensive use of Cremophor as a solubilizing agent in clinical formulations (e.g., with cyclosporin A and teniposide), its pharmacokinetics in humans have not previously been studied. The chemical inertness and lack of spectral properties of Cremophor have precluded the development of a specific assay, but the use of a bioassay circumvents these problems. It is recognized that Cremophor is a heterogeneous compound and that the bioassay measures only the MDR-modulating component(s). However, for the purpose of examining the potential role of Cremophor in reversing MDR, a bioassay is preferable to a chemical assay, since the relevant pharmacologic effect is being assessed more directly. In this study, we have used a bioassay to measure plasma Cremophor concentrations in patients with ovarian cancer who were randomly assigned to receive paclitaxel as 3-, 6-, and 24-hour infusions.

Patients and Methods

Study Design

Eligibility criteria for this study included histologically confirmed epithelial ovarian cancer, at least one prior platinum-containing chemotherapy regimen but no more than three prior regimens, an age of 75 years or less, a performance status of 0-2, at least one prior platinum-containing chemotherapy, at the end of the infusion, and then at 0.5, 1, 2, 3, 6, and 24 hours following the infusion; for the 6-hour infusion, at 1, 2, and 3 hours during the infusion, at the end of the infusion, and then at 0.5, 1, 2, 3, 6, 18, and 18 hours following the infusion; for the 24-hour infusion, at 3, 6, 12 hours during the infusion, at the end of the infusion, and then at 0.5, 1, and 2 hours following infusion.

Cremophor Assay

The multidrug resistant CEM/VLB100 human T-cell leukemia cell line was maintained at 37°C in Eagle's minimum essential medium (α-modification: Gibco, Melbourne, Australia) supplemented with 10% fetal calf serum and 100 ng/mL vinblastine (F. H. Faulding Pharmaceuticals, Adelaide, Australia). Cremophor EL was purchased from BASF Fine Chemicals (Melbourne). The assay was performed as described previously with minor modifications (9,11). Briefly, this assay measures the ability of plasma samples to modulate MDR in vitro. Our initial experiments (9,11) had indicated that a Cremophor concentration of 1 μL/mL or more was sufficient for maximum (100%) MDR reversal in vitro. However, repeat experiments have shown that a concentration of 1 μL/mL is sufficient for 50% reversal of MDR and that concentrations of 1.5-2.0 μL/mL are required for maximum reversal (unpublished observations). Inhibition of the Pgp drug-efflux pump by Cremophor results in increased intracellular daunorubicin accumulation. To measure Cremophor in the plasma from patients, CEM/VLB100 cells (5 x 10^6) were incubated at 37°C for 1 hour with 0.5 mL plasma and 0.5 mL growth medium containing 2 μg/mL daunorubicin. Intracellular daunorubicin fluorescence was measured by means of flow cytometry (FACStar Plus cell sorter: Becton Dickinson, NJ, or Coulter Epics Profile II; Coulter Electronics, Miami, FL). All measurements were done in tripliate. The within-day coefficient of variation was less than 10%, the interassay coefficient of variation was 14%, and the lower limit of detection was 0.05 μL/mL. Pretreatment plasma was used to derive a 6-point standard curve for the effect of Cremophor on intracellular daunorubicin fluorescence. The increase in equilibrium intracellular daunorubicin fluorescence in assays containing post-treatment plasma gave a measure of the Cremophor concentration.

Pharmacokinetics

Pharmacokinetic parameters for Cremophor in plasma were calculated with standard model-independent equations, using the SIPHAR/WIN computer package (SIMED; Creteil, France). The area under the plasma concentration versus time curve (AUC) was estimated with the trapezoidal rule and was extrapolated to infinity (AUC_{inf}) for the 3- and the 6-hour infusions only. Since the bioassay used in this study measures the pharmacologic effect of Cremophor rather than a defined chemical species, the term "apparent AUC" is applied to this parameter. The terminal elimination half-life was calculated by linear regression of the final three to four points on the log-linear concentration-time profile. Total clearance was calculated as dose/ AUC_{inf} and was normalized to body surface area.

Statistical Methods

A comparison of the peak Cremophor concentrations for the three infusion groups was made by use of analysis of variance for crossover trials (12). The Glim statistical package (13) was used to carry out the analysis. Two-tailed significance levels have been reported, with no correction for multiple comparisons. Results were deemed to be significant if the P value was less than 0.05.

Results

Patient Characteristics

Eleven women with epithelial ovarian cancer had plasma Cremophor concentrations assayed after 3-, 6-, and 24-hour infusions of paclitaxel. The median patient age was 59 years (range, 35-67 years). Ten patients received paclitaxel at a dose of 175 mg/m^2, and one received the drug at a dose of 135 mg/m^2.

Plasma Cremophor Pharmacokinetics

With 175 mg/m^2 paclitaxel, the total amount of Cremophor delivered was 23.5 ± 2.4 mL (mean ± standard deviation [SD]; n = 10). The mean peak Cremophor concentrations for the 3-, 6-, and 24-hour infusions were 1.47, 1.24, and 0.65 μL/mL, respectively (Fig. 1). There was a significant difference between the three groups (P<.0001). In particular, both the 3- and the 6-hour results were significantly higher than the 24-hour results (P< .0001), and the 3-hour results were higher than the 6-hour results (P = .04). The peak plasma Cremophor concentration was 1 μL/mL or more in eight of 10 patients during both the 3- and the 6-hour infusions. Only one patient achieved a peak level of 1 μL/mL or more during the 24-hour infusion. The patient who received paclitaxel at a dose of 135 mg/m^2 achieved a plasma Cremophor concentration of 1 μL/mL or more only with the 3-hour infusion. No patient attained a
plasma Cremophor concentration of 2 μL or more, although six patients achieved a concentration of 1.5 μL or more during the 3-hour infusion.

The mean plasma Cremophor concentrations in 10 patients receiving 175 mg/m² paclitaxel for each infusion duration are shown in Fig. 2. By 30 minutes into the 3-hour infusion, the eight patients at this dose who achieved at least 1 μL/mL Cremophor were already above this level, suggesting that high Cremophor concentrations are reached rapidly with 3-hour paclitaxel infusions. Furthermore, the concentration–time profile for the 3-hour infusion was unusual in that the mean plasma Cremophor concentrations decreased during the infusion period. For the 6-hour infusion, the mean plasma Cremophor concentration was above 1 μL/mL by 2 hours into the infusion. In the eight patients who reached plasma concentrations of 1 μL/mL during the 3-hour infusion, the total time that Cremophor concentrations were greater than 1 μL/mL was 8.9 ± 5.0 hours (mean ± SD; range, 4.1-15.6 hours). For the 6-hour infusion, the total time above 1 μL/mL was 10.2 ± 9.0 hours (range, 0.3-21.9 hours; n = 8). Thus, interpatient variability in the duration of plasma Cremophor concentrations above 1 μL/mL is high. The Cremophor concen-
trations during the 24-hour infusion period remained below the level required for at least 50% reversal of MDR in vitro.

Table 1 summarizes the pharmacokinetic parameters for Cremophor in nine patients who received 175 mg/m² paclitaxel. One patient was excluded from the pharmacokinetic analysis because of insufficient data on the terminal-elimination phase during the 6-hour infusion. The reported terminal-elimination half-lives must be considered as estimates only, since the durations of sampling were not three times the calculated half-life values. The pharmacokinetics were nearly identical for both the 3- and the 6-hour infusions, and, despite the long estimated half-lives, the peak plasma concentration for each infusion time was reached prior to the end of the infusion. In contrast, Cremophor concentrations during the 24-hour infusion period were highest at the end of the infusion.

Discussion

In this study, we have confirmed our previous finding that 3-hour infusions of paclitaxel produce plasma Cremophor concentrations at the end of infusion that are sufficient for substantial reversal of MDR in vitro (11). Moreover, Cremophor concentrations are maintained at levels that could potentially reverse MDR for prolonged periods of time both during and following 3- and 6-hour infusions in most patients. However, interpatient variability was found to be high, and no patient achieved a plasma Cremophor concentration of 2 µL/mL or more (i.e., a concentration sufficient for maximum MDR reversal in vitro). Since Cremophor is a known inhibitor of Pgp and since paclitaxel is transported by this efflux pump, sustained Cremophor concentra-

tions achieved with paclitaxel infusions of up to 6 hours in duration may potentially reverse MDR in tumors that overexpress Pgp. The Cremophor concentrations achieved during 24-hour infusions of paclitaxel were not adequate for ample MDR reversal in vitro. However, it is not known whether Cremophor concentrations capable of reversing MDR in vitro are capable of reversing MDR in patients. Furthermore, the relative importance of Pgp-associated MDR in the development of intrinsic or acquired clinical resistance to paclitaxel is not known. A preliminary report (14) has suggested that there is an association between the response to 24-hour infusions of paclitaxel and low to nonexistent levels of Pgp expression in patients with metastatic breast cancer.

The optimum infusion duration for paclitaxel is controversial. Significant antitumor activity has been reported in clinical trials with infusion durations ranging from 1 to 96 hours (2-4). A randomized trial that compared 3- and 24-hour infusions in patients with relapsed ovarian cancer found no difference in response rates or survival (2). Experimental data do suggest that a more prolonged exposure may be more efficacious against some cell lines (15). More prolonged exposure can also decrease the degree of resistance in MDR cell lines exposed to paclitaxel in vitro (15). However, complete reversal does not occur, unlike the situation with Cremophor, where complete reversal of paclitaxel resistance in Pgp-expressing human breast cancer cell lines has been demonstrated (16). It is noteworthy that responses have been seen with 96-hour infusions in patients with breast cancer who were refractory to 3-hour infusions (17). The relationship between Pgp expression and response in that study is not known. It is possible that more prolonged infusions may be more efficacious for tumors that do not overexpress Pgp, whereas short infusions that attain high concentrations of Cremophor may be better in treating tumors that overexpress this protein. Randomized trials that include an analysis of Pgp expression will be required to resolve this issue.

Our finding of sustained high Cremophor concentrations with short infusions of paclitaxel also has implications for the interpretation of clinical trials of other MDR modulators that have been used with paclitaxel (e.g., 3-hour infusions of paclitaxel in combination with R-verapamil or the cyclosporin SDZ PSC 833 (18,19)). In such studies, the effect of two potential modulators is being examined. Additional benefit would be expected only if more complete inhibition of Pgp than can be achieved with Cremophor alone translates into improved reversal of MDR.

The pharmacokinetic parameters determined for Cremophor in the present study are effectively those of the MDR-active component only. This may explain the apparent nonlinear pharmacokinetics suggested by the unusual shape of the plasma concentration versus time curve during the 3- and the 6-hour infusions, whereby the maximum Cremophor concentration was reached prior to the end of the infusion, despite the estimated elimination half-life of 26 hours. The maximum concentration and the AUC achieved with the 24-hour infusion were approximately half the values determined with the shorter infusions. Although the metabolism and excretion of Cremophor are unknown, the pharmacokinetics suggest that the MDR-active component is a relatively low clearance compound.

Cremophor pharmacokinetics following paclitaxel administration are of inter-

Table 1. Plasma Cremophor pharmacokinetics (mean ± standard deviation, n = 9) in patients receiving infusions of paclitaxel at 175 mg/m².

<table>
<thead>
<tr>
<th>Infusion duration, h</th>
<th>Tmax, h</th>
<th>Cmax, µL/mL</th>
<th>t½, h</th>
<th>AUC (0-4 h) µL h/mL</th>
<th>AUC (0-∞) µL h/mL</th>
<th>Cl (0-∞) mL/h/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1 ± 0.1</td>
<td>1.3 ± 0.6</td>
<td>1.5 ± 0.3</td>
<td>26.1 ± 8.8</td>
<td>23.9 ± 5.3</td>
<td>47.3 ± 16.1</td>
<td>361 ± 183</td>
</tr>
<tr>
<td>6.1 ± 0.1</td>
<td>4.6 ± 1.8</td>
<td>1.3 ± 0.3</td>
<td>26.4 ± 8.0</td>
<td>20.9 ± 7.1</td>
<td>44.9 ± 21.1</td>
<td>402 ± 210</td>
</tr>
<tr>
<td>24.9 ± 1.8</td>
<td>24.9 ± 1.8</td>
<td>0.7 ± 0.2</td>
<td>n.c.</td>
<td>11.7 ± 3.4</td>
<td>n.c.</td>
<td>n.c.</td>
</tr>
</tbody>
</table>

* Total amount of Cremophor delivered to an individual patient was the same for each infusion duration (23.9 ± 2.3 mL).
† Tmax = time to achieve maximum plasma concentration; Cmax = maximum plasma concentration; t½ = terminal elimination phase half-life; AUC = area under the plasma concentration versus time curve; Cl = clearance; t = time; n.c. = not calculated.
‡ The final sample collection time depended on the infusion duration, and was 27, 24. and 27 hours for the 3-, 6-, and 24-hour infusions, respectively.
est for several reasons in addition to the potential for MDR reversal. We have recently reported that Cremophor, when given as an MDR modulator with doxorubicin, results in an increase in the AUC of doxorubicin and doxorubicinol (20,21). Cremophor may also alter the pharmacokinetics of etoposide (22). The altered pharmacokinetics of other drugs by Cremophor has significant implications for combination chemotherapy with paclitaxel. For example, Cremophor has been implicated in the high doxorubicin and doxorubicinol concentrations seen when 3-hour infusions of paclitaxel were combined with doxorubicin (23), and this may have contributed to the 21% incidence of cardiac failure in that trial (24). It is also possible that Cremophor may affect the pharmacokinetics of paclitaxel and may contribute to the non-linear pharmacokinetics seen with short infusions (25). Cremophor has also been demonstrated to antagonize the cytotoxicity of paclitaxel in cell lines that do not overexpress Pgp (26). It will now be possible to determine the effect of the clinically relevant Cremophor concentrations achieved with short and prolonged infusions on paclitaxel cytotoxicity in vitro. Cremophor also alters human plasma lipoproteins, and these effects are more marked with 3-hour infusions than with 24-hour infusions of paclitaxel (27). The effect on lipoproteins is due to a component of Cremophor that is different from the one that modulates MDR.

The efficacy of paclitaxel against cancer resistant to other chemotherapeutic agents cannot be explained solely by reversal of MDR by Cremophor, since prolonged infusions also have activity in this setting despite low Cremophor concentrations. Clinical drug resistance may be caused by several mechanisms, with the predominant mechanism depending on a number of factors, including tumor type and previous chemotherapy. Nevertheless, we have demonstrated in this study that short infusions of paclitaxel result in sustained plasma Cremophor concentrations that are sufficient to modulate MDR, thus having the potential to contribute to the efficacy of paclitaxel when Pgp-associated MDR is the limiting mechanism for response. The ultimate significance of our findings awaits further studies that are required to define the role of Pgp-associated MDR in clinical paclitaxel resistance.

References

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Notes

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REPORTS 1301