Effects of α₁-Acid Glycoprotein on the Clinical Pharmacokinetics of 7-Hydroxystaurosporine

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ABSTRACT

Objective: UCN-01 (7-hydroxystaurosporine) is a small molecule cyclin-dependent kinase modulator currently under clinical development as an anticancer agent. In vitro studies have demonstrated that UCN-01 is strongly bound to the acute-phase reactant α₁-acid glycoprotein (AAG). Here, we examined the role of protein binding as a determinant of the pharmacokinetic behavior of UCN-01 in patients.

Experimental Design: Pharmacokinetic data were obtained from a group of 41 patients with cancer receiving UCN-01 as a 72-hour i.v. infusion (dose, 3.6 to 53 mg/m²/day).

Results: Over the tested dose range, total drug clearance was distinctly nonlinear (P = 0.0076) and increased exponentially from 4.33 mL/hour (at 3.6 mg/m²/day) to 24.1 mL/hour (at 54 mg/m²/day). As individual values for AAG increased, values for clearance decreased in a linear fashion (R² = 0.264; P = 0.0008), although the relationship was shallow, and the data showed considerable scatter. Interestingly, no nonlinearity in the unbound concentration (P = 0.083) or fraction at the peak plasma concentration of UCN-01 was apparent (P = 0.744).

Conclusion: The results suggest the following: (1) that extensive binding to AAG may explain, in part, the unique pharmacokinetic profile of UCN-01 described previously with a small volume of distribution and slow systemic clearance, and (2) that measurement of total UCN-01 concentrations in plasma is a poor surrogate for that of the pharmacologically active fraction unbound drug.

INTRODUCTION

Protein kinases represent key signaling molecules important in growth control and carcinogenesis, among other cellular signaling systems. Two families of protein kinases can be defined, based on phosphorylation of tyrosine and serine/threonine (1), and two general strategies to design pharmacological modulators of protein kinase function have been used: (1) by targeting the ATP-binding site of the kinases, and (2) by targeting non-ATP site-mediated aspects of kinase function. The staurosporine derivative UCN-01 (7-hydroxystaurosporine) is an example of an ATP-site (or ATP-enzyme complex)-directed protein kinase antagonist (2) and is currently being evaluated in clinical trials for the treatment of cancer (3, 4). It was originally recognized as a potent and selective antagonist of protein kinase C. Subsequent studies revealed greater selectivity for the α, β, and γ isofroms of protein kinase C. In addition, it was found to have potent antiproliferative effects in a way that was not clearly related to inhibition of protein kinase C. Among the cellular targets that have emerged as sensitive to UCN-01 are chk1 and chk2 DNA damage-dependent checkpoint kinases, phosphatidylinositol-dependent kinase I (PDK1), and pathways leading to cyclin-dependent kinase activation (reviewed in refs. 3, 4).

Very little information is available on the metabolic fate of UCN-01. In laboratory animals treated i.v. with [3H]UCN-01, the radioactivity was mainly excreted in feces, reaching 96.0% of the radioactivity dose in rats and 78.4% in dogs up to 168 hours after injection. Because the biliary excreted radioactivity was 67.2% over 48 hours in bile duct-canulated rats, most of the radioactivity excreted in feces was from biliary radioactivity (5). Although little UCN-01 appears to be excreted as unchanged drug, the chemical structures and biological activity of metabolites are still unknown.

UCN-01 was shown previously to bind extensively in vitro to the human acute-phase reactant AAG (α₁-acid glycoprotein; Kₐ = 799 × 10⁴ L/mol) in a concentration-dependent manner, with high AAG concentrations being associated with a decrease of the unbound drug fraction (6) and altered pharmacodynamic effects in vitro (7). In a Phase I clinical study, the disposition of UCN-01 was shown to be very distinct from other PDK1 modulators and characterized by a small volume of distribution (~12 L) and an exceptionally slow systemic clearance (~17 mL/hour; ref. 8). Here, we describe the development and evaluation of a pharmacokinetic model, which includes the AAG concentration and unbound drug elimination to additionally clarify the role of AAG in the clinical pharmacology of UCN-01.

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MATERIALS AND METHODS

Chemicals. UCN-01 reference material was provided by Kyowa Hakko Kogyo Co. (Tokyo, Japan), and formulated for clinical use by the Developmental Therapeutics Program of the National Cancer Institute (Bethesda, MD). Ammonium acetate, the high-performance liquid chromatography internal standard umbelliferone, and human AAG were purchased from Sigma Chemical Co. (St. Louis, MO). Acetonitrile was obtained from J. T. Baker (Phillipsburg, NJ). All of the solvents and chemicals were of analytical reagent grade or better. Deionized water was obtained from a Milli-Q-UF system (Millipore, Milford, MA) and used throughout in all of the aqueous solutions. Drug-free (blank) human plasma originated from Pittsburgh Blood Plasma, Inc. (Pittsburgh, PA).

Patient Selection and Treatment. The current pharmacokinetic analysis was performed on samples obtained from patients with a histologically proven malignant solid tumor or lymphoma refractory to standard therapy, who participated in a Phase I evaluation of UCN-01 given as an i.v. infusion. Specific inclusion and exclusion criteria and detailed toxicological and response data for this cohort of patients were described in detail previously (8). UCN-01 was given as a 72-hour continuous infusion (mean, 71.92 hours; range, 44.58 to 80.67 hours) at previously (8). UCN-01 was given as a 72-hour continuous infusion (mean, 71.92 hours; range, 44.58 to 80.67 hours) at

Blood Sampling and Analysis. Blood samples of ~7 mL were collected in heparin-containing tubes at the following time points: immediately before drug administration; at 4, 12, 24, and 48 hours after the start of drug administration; at 5 to 10 minutes before the end of infusion; and at 0.5, 1, 2, 3, 6, 12, 24, 48, 216, 264, 380, and 576 hours after the end of infusion. In a limited number of patients, additional samples were obtained at 640, 800, 960, 1130, 1300, 1640, 1800, 2140, 2500, and 2650 hours after the end of infusion. After collection, specimens were immediately centrifuged at 3,000 × g for 10 minutes to separate the plasma supernatant, which was stored at −70°C until the time of analysis. Concentrations of total UCN-01 in plasma were determined by a validated ultracentrifugation technique, as described elsewhere (6). AAG concentrations in pretreatment plasma samples were measured before storage in the freezer using a standard turbidimetric assay.

Pharmacokinetic Analysis. Plasma concentration-time data obtained for total UCN-01 administered as a 72-hour infusion were analyzed using a standard two-compartment linear model (Fig. 1A), using equations A and B:

\[
\frac{dC_1}{dt} = k_0 + k_{31} \times C_2 - k_{12} \times C_1 - k_8 \times C_1 \\
\frac{dC_2}{dt} = k_{12} \times C_1 - k_{21} \times C_3
\]  

(A) \hspace{1cm} (B)

An alternative model based on a two-compartment model, which incorporates a protein-binding factor in the central compartment (Fig. 1B) was evaluated, based on equations C, and D:

\[
\frac{dC_1}{dt} = k_0 + k_{31} \times C_2 - k_{12} \times C_1 - k_8 \times C_1 - Cu \\
C_u = \left[ \left(1 + k + npk \times C_1 \right)^2 + 8k_8 \times C_1 \right]^{\frac{1}{2}}
\]  

(C) \hspace{1cm} (D)

Fig 1 Pharmacokinetic models used to describe concentration-time profiles of UCN-01 given as a 72-hour i.v. infusion to patients with cancer at dose levels ranging 3.6 to 53 mg/m²/day. A, a standard two-compartment model and (B) a two-compartmental model, involving protein binding in the central compartment and elimination of unbound drug.
In the equations, $k_{12}$ and $k_{21}$ are the intercompartmental rate constants, and $k_e$ is the elimination rate constant of UCN-01. It was assumed that only unbound UCN-01 in the central compartment (Cu) was eliminated (10). In equation (D), $P$ denotes the AAG concentration in patient plasma samples immediately before drug administration, $k_a$ is the association constant for the nonsaturable, nonspecific binding sites of UCN-01 to AAG, $k$ is the association constant for the nonsaturable, nonspecific binding, and $n$ the number of specific binding sites per molecule (11). Values for $k_a$ (i.e., $8 \times 10^8$ L/mol), $n$ (i.e., 0.721), and $k$ (i.e., $1 \times 10^9$ L/mol) were reported previously (6) and were used as constants in the equations. The pharmacokinetic analysis was performed using a weighted, nonlinear least-squares analysis as implemented in Adapt II release 4 (Biomedical Simulations Resource, Los Angeles, CA). Model discrimination was guided by inspection of the weighted sum of squares and the coefficient of variation of the fitted pharmacokinetic parameters, and by the Akaike information criterion. The peak concentration was put on par with the observed UCN-01 concentration obtained immediately before the end of infusion. The fraction unbound UCN-01 (fu) was calculated as the ratio of Cu and the total drug concentration and expressed as a percentage.

**Statistical Considerations.** Pharmacokinetic parameters are reported as mean values ± SD, unless stated otherwise. The effect of dose on the pharmacokinetics parameters clearance, volume of distribution, half-life, and fraction unbound drug was evaluated by the Kruskal-Wallis one-way ANOVA statistic followed by a Dunn’s test to determine group differences. Total variability in pharmacokinetic parameters was apparent at any dose level (coefficient of variation, up to 150%) and might, in part, be related to the long half-life of UCN-01 relative to the sample collection time period. Plasma concentrations of total UCN-01 increased gradually during infusion but failed to reach steady state by 72 hours, typical for drugs with a long terminal half-life. The initial disposition phase of total UCN-01 was characterized by a half-life of 6.80 ± 9.43 hours (range, 0.152 to 51.8 hours), whereas the terminal biological half-life was 749 ± 645 hours (range, 199 to 3,780 hours). The mean area under the plasma concentration-time curve extrapolated to infinity increased from 7,460 mg.hour/L at a dose of 3.6 mg/m²/day to 26,140 mg.hour/L at 53 mg/m²/day. Over the tested dose range, total drug clearance was also distinctly nonlinear ($P = 0.0076$) and increased exponentially from 4.13 mL/hour at 3.6 mg/m²/day to 24.1 mL/hour at 54 mg/m²/day (Fig. 3), whereas the steady-state volume of distribution increased linearly with dose ($R^2 = 0.442; P = 0.01$) from 0.113 L to 0.276 L. It is

![Fig. 3](https://example.com/fig3.png)
Table 1  Summary of pharmacokinetic parameter estimates

<table>
<thead>
<tr>
<th>Dose (n) (mg/m²/day)</th>
<th>Cmax (mg/L)</th>
<th>AUC (mg-h/L)</th>
<th>CL (mL/h)</th>
<th>Vc (L)</th>
<th>T1/2,z (h)</th>
<th>fu (%)</th>
<th>AAG (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.6 (3)</td>
<td>2.77 ± 0.813</td>
<td>7,460 ± 4,940</td>
<td>4.13 ± 3.79</td>
<td>2.15 ± 1.86</td>
<td>2310 ± 1670</td>
<td>0.934*</td>
<td>243 ± 277†</td>
</tr>
<tr>
<td>6 (3)</td>
<td>5.34 ± 0.622</td>
<td>5,480 ± 3,460</td>
<td>8.34 ± 4.60</td>
<td>3.61 ± 2.28</td>
<td>874 ± 472</td>
<td>0.666*</td>
<td>130 ± 170†</td>
</tr>
<tr>
<td>12 (3)</td>
<td>9.08 ± 2.15</td>
<td>16,150 ± 19,240</td>
<td>9.10 ± 6.43</td>
<td>3.89 ± 1.07</td>
<td>689 ± 320</td>
<td>1.48*</td>
<td>135 ± 69.3</td>
</tr>
<tr>
<td>17 (3)</td>
<td>12.4 ± 1.52</td>
<td>9,380 ± 1,690</td>
<td>11.0 ± 2.06</td>
<td>5.31 ± 2.59</td>
<td>850 ± 117</td>
<td>0.867 ± 0.751</td>
<td>155 ± 60.3</td>
</tr>
<tr>
<td>24 (3)</td>
<td>15.6 ± 5.51</td>
<td>8,910 ± 3,360</td>
<td>17.6 ± 6.07</td>
<td>1.70 ± 0.059</td>
<td>511 ± 300</td>
<td>1.06 ± 0.162</td>
<td>100 ± 20.0</td>
</tr>
<tr>
<td>34 (8)</td>
<td>16.3 ± 5.41</td>
<td>10,810 ± 3,460</td>
<td>20.4 ± 7.97</td>
<td>3.83 ± 2.00</td>
<td>605 ± 312</td>
<td>1.17 ± 0.685</td>
<td>147 ± 56.0</td>
</tr>
<tr>
<td>42.5 (10)</td>
<td>17.7 ± 4.48</td>
<td>13,620 ± 10,340</td>
<td>23.5 ± 11.8</td>
<td>3.49 ± 2.41</td>
<td>612 ± 370</td>
<td>1.24 ± 0.604</td>
<td>151 ± 23.9</td>
</tr>
<tr>
<td>53 (8)</td>
<td>19.1 ± 6.85</td>
<td>26,140 ± 38,940</td>
<td>24.1 ± 12.8</td>
<td>1.64 ± 0.766</td>
<td>509 ± 118</td>
<td>0.829 ± 0.270</td>
<td>130 ± 51.8</td>
</tr>
</tbody>
</table>

NOTE. Data were obtained using a linear two-compartment open model and are expressed as mean value ± SD.
Abbreviations: Cmax, peak concentration; AUC, area under the plasma concentration-time curve; CL, clearance; Vc, central volume of distribution; T1/2,z, half-life of the terminal disposition phase; fu, fraction unbound UCN-01 at peak concentration; AAG, α1-acid glycoprotein.

* fn = 1.
† fn = 2.

noteworthy that the steady-state volume of distribution is probably not well estimated because of the relatively sparse sampling design. The dependence of clearance on UCN-01 dose occurred without similar changes in the estimated terminal disposition half-life (P = 0.31). Furthermore, pharmacokinetic parameters for unbound UCN-01 were independent of the dose administered (P = 0.083; Fig. 4), consistent with linear pharmacokinetics. This suggests that the nonlinearity in clearance does not arise from factors associated with saturation of excretory routes.

Values for AAG in serum were available from only 39 patients and showed 4-fold variation with a median value of 132 mg/dL (range, 63 to 277 mg/dL), similar to previously reported estimates (12). The clearance of total UCN-01 was significantly related to AAG concentration (Fig. 5); as the AAG concentration increased, values for clearance decreased linearly (R² = 0.264; P = 0.0008), although the relationship was shallow, and the data showed considerable scatter. A similar, albeit weaker, relation was observed with the fraction unbound UCN-01 (fu; data not shown). Despite the notion that models of protein binding are inherently nonlinear, the equation of the straight line relating fu and AAG was well estimated as: fu = 1.7285–0.0045 × AAG using the 29 available paired observations in this data set. The y-intercept, the estimated value of fu when AAG is zero, was 1.7285 ± 0.3103, with a slope, the estimated change in fu per unit change in AAG, of –0.0045 ± 0.0021 (R² = 0.149; P = 0.0465). The observed plasma concentrations of UCN-01 were well described by both the standard two-compartment model as well as the model that incorporates individual AAG levels, although no significant advantage was noted for the latter model based on the Akaike information criterion (P > 0.05). This suggests model misspecification for the AAG model and, therefore, nonlinear protein binding could not explain the nonlinearity in the pharmacokinetic model.

DISCUSSION

The standard analytical methods for measuring concentrations of drugs determine drug bound to plasma proteins as well as unbound drug dissolved in plasma water. For this reason, the relationship between total drug concentration in plasma and treatment outcome (i.e., toxicity and efficacy) will only be good if the degree of binding of the agent is constant over time in the clinically relevant concentration range, if so little drug is protein bound that changes in binding make insignificant changes in unbound concentration, and/or if the off-rate of the drug from the binding protein is sufficiently high. Previous in vitro and animal studies demonstrated that the investigational anticancer drug UCN-01 is extensively bound to AAG (6, 10, 13, 14), an acute-phase reactant, the concentration of which is highly variable in cancer patients (12). The current data demonstrate that AAG concentration is a significant predictor of the unbound drug fraction that is directly available for cellular partitioning and disposition processes. In addition, similar to recently pub-
lished data for the anticancer drugs docetaxel (15), docosahexaenoic acid-paclitaxel (16), and imatinib (17), we found that increased levels of AAG were associated with a significant reduction of total drug clearance.

This present study also demonstrates a striking dose dependence in UCN-01 plasma pharmacokinetics in cancer patients, which contrasts previous findings from dose-response studies performed in dogs, wherein the clearance was independent over an 8-fold dose range of UCN-01 given over 24 hours (i.e., 0.81 to 6.48 mg/kg; refs. 5, 18). In our patients, a 30-fold increase in dose (from 3.6 to 54 mg/m²/day) was associated with only a 3.5-fold increase in systemic exposure to total UCN-01. Over the same dose range, both clearance and the volume of distribution at steady state demonstrated a >2-fold increase. The opposing effects of these two processes on drug elimination leave the half-life of the apparent terminal disposition phase of UCN-01 not significantly dependent on the drug dose administered. Clearly, this may have important clinical ramifications; if clinical outcomes are related to total drug exposure, then a simple percentage increase in dose will have much lesser impact on total drug exposure than would be expected with a behavior based on linear pharmacokinetics.

The dose-dependent pharmacokinetic behavior of UCN-01 in human patients most likely involves multiple nonlinear mechanisms, including saturation of binding to plasma proteins like AAG. Saturation of drug binding to plasma constituents, blood cells, and extravascular tissue leading to nonlinear pharmacokinetics is relatively common and has been well described for several drugs, including valproic acid (19). However, the phenomenon of a dose-dependent decrease in systemic exposure as seen here with i.v.-administered UCN-01 is highly unusual. Although the mechanistic basis for the dose-dependent pharmacokinetic profile observed here remains to be elucidated, it is likely that capacity-limited binding to AAG contributes to explaining this phenomenon. Support for this hypothesis comes from previous in vitro studies indicating that the fraction unbound UCN-01 dramatically increases with an increase in drug concentration at a fixed AAG concentration (6). Thus, the unbound concentration is expected to increase precipitously with an increase in dose to stoichiometric equivalence of UCN-01 to AAG concentrations. Some clinical implications from this process, particularly with respect to considerations of treatment schedule, are easily envisaged. Most importantly, it makes the use of total UCN-01 concentrations for therapeutic monitoring misleading, particularly if there is...
significant interindividual variability in the apparent binding constant (6). In addition, changes in AAG concentrations, which are known to occur in cancer patients, can result in time dependence of drug kinetics, especially with respect to total drug (20).

On the basis of the disposition characteristics of UCN-01, it was considered reasonable to evaluate a pharmacokinetic model that incorporates binding to plasma proteins in the central compartment, ignoring extravascular binding, to characterize concentration-time profiles for total drug. The hypothesis was that this model would accurately reflect the influence of AAG on the plasma concentrations of UCN-01. Specifically, we anticipated that as the AAG concentration increases, the fraction unbound of UCN-01 would decrease and, consequently, the volume of distribution at steady state and total clearance decrease. However, no nonlinearity in the unbound concentration was apparent. This is presumably because unbound UCN-01 is extensively metabolized and rapidly eliminated by hepatobiliary secretion through mechanisms that are nonsaturable in the tested range of unbound UCN-01 concentrations (5); indeed, simultaneous concentration-dependence for binding of drugs to plasma proteins with linear pharmacokinetics for the unbound concentration has been documented previously for several other drugs (reviewed in ref. 20). In addition, it cannot be excluded that large intra- and interindividual variability in AAG levels in cancer patients (21, 22) might have caused the lack of a statistically significant influence in the current model for UCN-01. To shed additional light on this issue and to provide a more robust test of nonlinear pathways of the distribution and excretion of UCN-01, inclusion of nonlinear protein binding equations will be implemented in future modeling to additionally improve the fit of pharmacokinetic models to the current data.

As with most anticancer drugs, the prescribed dose of UCN-01 is routinely calculated using body surface area as an independent variable, and the usefulness of this approach has been questioned recently (23, 24). Relative to the absolute clearance of UCN-01 (expressed in milliliters per hour), the interpatient variability in clearance decreased after correction for the body surface area of individual patients (expressed in mL/hour/m²), with coefficients of variation of 61.6% and 58.2%, respectively. In addition, a linear-regression analysis of absolute clearance of UCN-01 versus body surface area resulted in a significant relationship (R² = 0.134; P = 0.015), indicating that body surface area contributed to 13% of total interindividual pharmacokinetic variability. It is noteworthy that in the current data set AAG and body surface area were moderately interrelated (R² = 0.263; P = 0.0010) except for one outlier at the upper end of body surface area (i.e., 2.98 m²), suggesting that body surface area may not be an independent predictor of UCN-01 clearance. Nevertheless, the data confirm the previous notion that the use of body surface area-based dosing may be a preferred strategy for agents that are principally confined to the systemic circulation because of the known relationship between body size and blood volume (25, 26).

It is concluded that UCN-01 binding to plasma proteins is an important determinant of its pharmacokinetic behavior. The major fraction of the administered drug is sequestered by AAG, restricting the unbound concentration and affecting distribution and elimination pathways. These characteristics indicate that the systemic exposure to total drug is dictated by its capacity to bind AAG, suggesting that measurement of total drug concentrations in plasma is a poor surrogate for that of unbound drug (11). Additional analysis of the disposition of UCN-01 in individual cancer patients, with respect to the current findings, should be of great importance for our ability to better understand the role of the various biological factors that may influence the pharmacokinetic behavior and pharmacological actions of the compound and effects of other drugs administered concomitantly.

REFERENCES

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