Advances in Brief

Kinetics of the in vivo Interconversion of the Carboxylate and Lactone Forms of Irinotecan (CPT-11) and of Its Metabolite SN-38 in Patients

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Abstract

The kinetics of the in vivo interconversion of the carboxylate and lactone forms of the prodrg irinotecan, 7-ethyl-10-[(1-piperidino)-1-piperidino]carbonyloxycaamptothecin (CPT-11), and its active metabolite SN-38 were studied in five patients using a HPLC method that allows the simultaneous determination of all four compounds and detects any hydrolysis of lactones due to inadequate sample handling and storage. The apparent conversion of CPT-11 lactone to the carboxylate in vivo was rapid with a mean half-life of 9.5 min; the carboxylate became the predominant form of plasma CPT-11 soon after the end of the infusion. The ratio of the area under the plasma concentration-time curves of the lactone to total CPT-11 was 36.8 ± 3.5% (SD). In contrast, SN-38 was present predominantly as the lactone at all times and with little interpatient variability (lactone/total area under the plasma concentration-time curve ratio, 64.0 ± 3.4%). This may explain in part the promising activity of CPT-11 because CPT derivatives are active against their target, topoisomerase I, only in their lactone form.

Introduction

CPT is a potent antitumoral alkaloid first isolated from the Chinese shrub Camptotheca acuminata (1, 2). The lactone ring of CPT undergoes reversible and pH-dependent hydrolysis to yield a water-soluble carboxylate form (1, 3) which was used in early clinical trials of CPT but later abandoned because of severe and unpredictable toxicities (4). The interconversion of the two forms, which also occurs in vivo, is all the more problematic because only the lactone form can poison topoisomerase I (5), the target of CPT derivatives (6). Furthermore, it has been shown that the lactone and carboxylate forms are two kinetically distinct species and that the disposition of CPT derivatives in vivo is dependent on the form administered (7–9). Irinotecan, also known as CPT-11 (Fig. 1), is a promising water-soluble derivative of CPT which is cleaved in vivo to the extremely active SN-38 [7-ethyl-10-hydroxycaamptothecin] (10, 11). Recent pharmacokinetic studies of lactone and total CPT-11 and SN-38 have shown that the proportion of the AUC of SN-38 accounted for by the lactone form may vary significantly between patients (12, 13). Given that only the lactone form is active, this variability could worsen the unpredictability of clinical response and toxicity. However, the variability encountered in these studies could also be due to technical problems relating to: (a) the inability of measuring the carboxylate forms directly; (b) continuing hydrolysis of lactones in samples; and (c) absence of internal standard. For this reason, we recently developed a HPLC assay which overcomes these problems (14). In this paper, we describe the evaluation of the interconversion of the lactone and carboxylate forms of CPT-11 and SN-38 in patients using this method.

Materials and Methods

Chemicals. CPT was obtained from Sigma Chemical Co. (St. Louis, MO) and purified as described previously (14). Samples of the lactones of CPT-11 and SN-38, prepared by Yakult Honshua Co., Ltd. (Tokyo, Japan), were supplied by Bellon (Rhône-Poulenc Rorer, Neuly, France). Tetrabutyl ammonium phosphate was purchased from Waters (Millipore, Neuly, France) as a ready-to-use solution (PIC A). All other reagents were of analytical grade.

Patients. Five patients enrolled in ongoing phase I/II studies or treated on compassionate grounds were studied at varying stages of treatment. Irinotecan was administered in an infusion 90 min (patient C) or 30 min (all others) long every 3 weeks. The patients had satisfactory WHO/Eastern Cooperative Oncology Group scores (0 or 1) and liver and kidney function (total bilirubin <15 μmol/liter, creatinine <110 μmol/liter; and normal plasma transaminase levels). All were receiving treatment for metastatic adenocarcinoma of colorectal, cervical, or unknown origin. Informed consent of the experimental procedure was obtained prior to the experiments. The characteristics of these patients and of their respective treatments are presented in Table 1.

Sample Collection and HPLC Analysis. The simultaneous analysis of the carboxylate and lactone forms of CPT-11 and SN-38 has been described elsewhere and was used in this study with only slight modifications (14). Blood samples (3 ml) were withdrawn before and during the infusion and then at 5, 20, and 40 min and 1, 1.5, 2, 4, 6, and 24 h after the infusion. These were collected into heparinized tubes and briefly immersed into a dry ice/acetone bath. This was done rapidly with gentle agitation, taking care not to freeze the blood. The sample was then centrifuged for 3 min (4°C, 8000 × g) and 200 μl of plasma were added to an Eppendorf tube containing 400 μl of an ice-cold methanol:acetone mixture (50:50, v/v) and 0.2 μg of CPT. The tubes were vortexed briefly and centrifuged (3 min, 4°C, 8000 × g), and the supernatant was transferred to a new tube and stored in a −70°C freezer. Prior to analysis the samples were thawed and centrifuged, and aliquots (50 μl) were diluted with an equal volume of a mobile phase buffer just before injection onto the column (5–20 μl). Samples were always handled on ice to minimize continued lactone hydrolysis (15). Peak data were recorded and compared to standard samples prepared using fresh human plasma. Two standard curves were determined daily, one for the carboxylates and one for the lactones as described previously (14).

Separation of the peaks of interest was performed using a Waters Nova-Pak Radial-Pak C4 reverse-phase column preceded by a guard column containing the same packing (Guard-Pak and Nova-Pak; Waters, Millipore, France). The mobile phase consisted of 78% (v/v) of 0.075 M ammonium acetate buffer (pH 6.4) and 22% acetonitrile to which 1 vial of PIC A solution was added. The final concentration of tetrabutyl ammonium phosphate was 5 mM. The mobile phase was delivered isocratically at 1.5 ml/min. Fluorimetric detection (Hitachi F-1050; Merck, France) was carried out with excitation and emission wavelengths of 355 and 515 nm, respectively.

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To whom requests for reprints should be addressed, at Département de Biochimie Médicale, Université de Bordeaux II, 146 rue Léo Saignat, Bordeaux Cedex, 33076 France. The abbreviations used are: CPT, 2(5S)-camptothecin; CPT-11 (irinotecan), 7-ethyl-10-[(1-piperidino)-1-piperidino]carbonyloxycaamptothecin; AUC, area under the plasma concentration-time curve; Cl, total body clearance.
comparison of Akaike’s Criterion Index values obtained with different numbers of terms. The half-lives of the corresponding phases were determined as

$$t_{1/2} = \frac{0.693}{\lambda_i}$$  \hspace{1cm} \text{(C)}$$

In the case where the tissue uptake and elimination from the plasma compartment is identical for the two forms of CPT-11, it can be shown that the ratio of lactone to total CPT-11 in the plasma at a time $t'$ after the infusion, $R(t')$ is

$$R(t') = \left( R_1 - \frac{k_2}{k_{op} + k_{oc}} \right) e^{-k_{op} t'} + \frac{k_2}{k_{op} + k_{oc}}$$  \hspace{1cm} \text{(D)}$$

where $R_1$ is the ratio in the plasma at the end of the infusion and the $k_{op}$ and $k_{oc}$ are the rate constants of ring opening (hydrolysis) and closing, respectively. This equation was fitted to the ratio versus time profiles of the patients to estimate the exponent by the least-squares method mentioned above. The apparent in vivo half-life of hydrolysis was then calculated from the product of the reciprocal of the exponent and 0.693.

### Results

The plasma profiles of the two forms of CPT-11 were similar for the five patients studied. Typical examples are shown in Fig. 2. The concentrations of the lactone form of CPT-11 rose during the infusion and decreased very rapidly once this infusion was finished. A slower phase of elimination then became evident 10–20 min following the infusion. The plasma kinetics of this compound was best described when three exponential terms were used in Equation B. The corresponding half-lives are shown in Table 2. In contrast, the concentrations of the carboxylate form rose during the infusion and reached a maximum at approximately 1 h after the infusion, at which stage this form predominated. This difference in the kinetics of the two forms resulted in a “humped” profile for total CPT-11, which was particularly evident when the latter concentrations were plotted using a linear ordinate (Fig. 3). Rapidly, the interconversion of the two forms appeared to reach an equilibrium which was remarkably similar for the five patients with the lactone form of CPT-11 accounting for approximately 25–30% of the concentrations of total CPT-11 (Fig. 4). The AUC of the lactone form was found to represent 36.8 ± 3.5% of the total AUC. The apparent rate constant of interconversion ($k_{op} + k_{oc}$) estimated from the ratio data (Equation D) was 0.073 ± 0.034 (SD) min$^{-1}$, which corresponds to a mean half-life of 9.5 min.

The kinetics of SN-38 appeared to be very different with the concentrations of the lactone and carboxylate forms being relatively constant following infusion (Fig. 2A), with the exception of patient D (Fig. 2B), for whom the maximum plasma concentrations of SN-38 lactone occurred at the end of the perfusion and then decreased rapidly. The other four patients had maximum SN-38 lactone concentrations between 30 min and 4 h following the end of the infusion. Although the lactone form accounted for approximately 80% of total plasma SN-38 in the first few hours after the infusion, the AUC of the

### Pharmacokinetic Analysis

The AUC for each of the four compounds were determined by the trapezoidal method and extrapolated to infinity using the apparent terminal rate constant of elimination ($\lambda_i$). The first moment of the plasma concentration-time curve (AUMC) of CPT-11 lactone was estimated likewise. Model-independent values of the CL and volume of distribution at steady state ($Vd_{ss}$) of CPT-11 lactone were estimated from

$$CL = \frac{\text{Dose}}{\text{AUC}} \hspace{1cm} Vd_{ss} = \frac{\text{Dose} \cdot \text{AUMC}}{\text{AUC}^2} - \frac{\tau \cdot \text{Dose}}{2 \cdot \text{AUC}}$$  \hspace{1cm} \text{(A)}$$

where $\tau$ is the duration of the infusion. In addition, the plasma kinetics of the lactone form of CPT-11 was analyzed according to the exponential equation

$$C = \sum_{i=1}^{n} \frac{A_i}{\lambda_i^{\tau}} (e^{-\lambda_i \tau} - e^{-\lambda_i t})$$  \hspace{1cm} \text{(B)}$$

where $A_i$ and $\lambda_i$ are the $i$th coefficient and exponent, respectively, $\tau$ is the duration of the infusion and $t'$ is the time postinfusion ($t - \tau$) and has a value of zero when $t < \tau$. The fitting was carried out using a modified version of the nonlinear least-squares program of Yamaoka et al. (16) with a simplex algorithm. The weighting of the data used was $C^2$ where $C$ is the concentration measured at time $t$. The suitable number of exponents ($n$) was estimated by a

### Table 1 Characteristics of the patients studied

<table>
<thead>
<tr>
<th>Patient</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>69</td>
<td>52</td>
<td>60</td>
<td>58</td>
<td>69</td>
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<tr>
<td>Sex</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>F</td>
<td>F</td>
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<tr>
<td>Cycle no.*</td>
<td>7</td>
<td>11</td>
<td>3</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Dose rate as mg/m$^2$</td>
<td>350</td>
<td>500</td>
<td>300</td>
<td>350</td>
<td>350</td>
</tr>
<tr>
<td>Dose/mg/kg</td>
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<td>12.0</td>
<td>9.1</td>
<td>9.2</td>
<td>9.7</td>
</tr>
<tr>
<td>Total dose (mg)</td>
<td>637</td>
<td>1000</td>
<td>420</td>
<td>532</td>
<td>525</td>
</tr>
<tr>
<td>Duration of infusion (min)</td>
<td>30</td>
<td>30</td>
<td>90</td>
<td>30</td>
<td>30</td>
</tr>
</tbody>
</table>

* Number of times the patient had been given CPT-11 at the time of the study (inclusive of study).
SN-38 lactone accounted for 64.0 ± 3.4% of the total SN-38 AUC. As observed with CPT-11, the proportion of lactone to total SN-38 as a function of time was remarkably similar for all patients (Fig. 4).

Discussion

The HPLC methodology which we have developed recently (14) ensures that the compounds of interest have not undergone continued hydrolysis thanks to the use of CPT lactone both as an internal standard and as an indicator of sample degradation (none of the samples assayed in this study showed significant hydrolysis of CPT). This safeguard makes our technique ideal for studying the interconversion of the lactones and carboxylates of CPT-11 and SN-38. Although we have investigated only a small number of patients, having begun the study at the end of several existing phase I trials, we found surprisingly low variability of this interconversion despite the fact that the patients received different doses and were at differing stages of treatment. This is in contrast with the high variability in interconversion observed in recently published studies in which the proportion of lactone SN-38 to the total SN-38 AUC varied from 7 to 75% (13). Although our results need to be confirmed in a larger number of patients, they tend to indicate that part of the variability could be due to methodological problems. The reason for the observed predominance of the lactone form of SN-38 in plasma is not known but could be due to the differential formation, elimination, and protein binding of the two forms. Indeed, recent studies have shown that the rate of hydrolysis of SN-38 lactone in the presence of albumin is reduced in comparison with other CPT derivatives due to the preferential protein binding of the lactone form (17).

With the prodrug CPT-11, however, the carboxylate becomes the predominant form soon after the end of the infusion. In fact, the

<table>
<thead>
<tr>
<th>Table 2 Pharmacokinetics of the lactone and carboxylate forms of CPT-11 and SN-38 lactone in patients</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>AUC (μmol)</td>
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<table>
<thead>
<tr>
<th>Patient</th>
<th>CPT-11</th>
<th>SN-38</th>
<th>Kinetics of CPT-11 lactone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carb</td>
<td>Lact</td>
<td>Lact/tot</td>
</tr>
<tr>
<td>A</td>
<td>23.3 (1.8)(^b)</td>
<td>16.2 (3.8)</td>
<td>41.0</td>
</tr>
<tr>
<td>B</td>
<td>26.5 (10.2)</td>
<td>15.2 (6.8)</td>
<td>36.5</td>
</tr>
<tr>
<td>C</td>
<td>19.3 (4.3)</td>
<td>18.6 (10.3)</td>
<td>32.2</td>
</tr>
<tr>
<td>D</td>
<td>18.6 (10.3)</td>
<td>12.3 (15.7)</td>
<td>39.8</td>
</tr>
<tr>
<td>E</td>
<td>39.0 (8.5)</td>
<td>19.7 (4.1)</td>
<td>33.6</td>
</tr>
</tbody>
</table>

Mean ± SD:

- CPT-11: 36.8 ± 3.5
- SN-38: 64.0 ± 3.4
- Kinetics: 39.0 ± 9.6

\(^a\) Estimated using model-independent methods.

\(^b\) Carb, carboxylate; Lact, lactone; tot, total.

\(^c\) Percentage of total AUC which is accounted for by the extrapolation to infinity.

Fig. 3. Plasma concentration versus time profile of the carboxylate (○), lactone (●), and total forms (□) of CPT-11 in patient C plotted using a linear ordinate. Note the shoulder or hump which appears in the profile of total CPT-11 as a result of the rising concentrations of CPT-11 carboxylate. . . . . duration of infusion (90 min).

Fig. 2. Plasma concentration versus time profile of the carboxylates and lactones of CPT-11 (○ and ●, respectively) and SN-38 (□ and ■, respectively) in patients B (A) and D (B). . . . . duration of the infusion, 30 min in both instances.
apparent half-life of the interconversion of CPT-11 in vivo estimated in this study is less than a one-third of that reported for in vitro incubations in the presence of human serum albumin (17). This suggests that the simplifying assumption of equivalent disposition of the two forms of CPT-11 in Equation D is not correct and that there is a preferential uptake and/or metabolism of the lactone form of CPT-11 at early times thereby accelerating the predominance of the carboxylate form. It is clear, therefore, that continued research into the kinetics of CPT-11 disposition is not possible without quantification of both forms.

The extent of the hydrolysis of CPT analogues in vivo appears to vary enormously. For example, a preliminary pharmacokinetic study of 9-aminocamptothecin administered as a 72-h infusion has revealed that the lactone form accounts for only 3–7% of total drug at steady state (18). It is possible that the extent and rapidity of conversion to the inactive carboxylate in vivo could be an important factor in determining the antitumoral activity of CPT derivatives. In the study presented here, the lactone was found to be the predominant form of SN-38 in the plasma of five patients in a reproducible manner. This interesting property of SN-38, which could partly explain the promising activity of the produg CPT-11, should be investigated further.

References
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