Pharmacokinetics of SN-38 [(+)-(4S)-4,11-Diethyl-4,9-dihydroxy-1H-pyra[3',4':6,7]-indolizino[1,2-b]quinoline-3,14(4H,12H)-dione], an Active Metabolite of Irinotecan, after a Single Intravenous Dosing of 14C-SN-38 to Rats

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Irinotecan (CPT-11) is a camptothecin derivative used for the treatment of cancer. It is a prodrug that is metabolized to its active form, SN-38 [(+)-(4S)-4,11-diethyl-4,9-dihydroxy-1H-pyra[3',4':6,7]-indolizino[1,2-b]quinoline-3,14(4H,12H)-dione]. To clarify the pharmacokinetic difference between CPT-11 and SN-38, the plasma levels, tissue distribution and excretion of SN-38 were investigated after dosing rats with 14C-labeled SN-38.

The plasma radioactivity showed bi-exponential decay with a terminal half-life of 9.91 h. TLC separation revealed that the plasma radioactivity consisted mainly of SN-38 at 5 min after dosing; however, it was soon replaced with SN-38 glucuronide (SN-38 Glu) and an unknown metabolite (M-2). The half-life of unchanged SN-38 after dosing with SN-38 was about 7 min, which was much shorter than that after dosing with CPT-11 (2.8 h) as reported previously. Its radioactivity was excreted mainly in feces (70.0% within 168 h), and biliary excretion (64.1% within 48 h) could account for the fecal excretion. The major component of urinary and biliary radioactivity was found by TLC to be SN-38. Whole body autoradiograms revealed that the tissue distribution of the radioactivity was low except in the liver and kidney. The radioactivity decreased rapidly and little was found in the body 24 h after dosing.

In conclusion, SN-38 was excreted rapidly from bile and showed poor tissue distribution. These characteristics lead to a shorter SN-38 half-life, more so than dosing with CPT-11.

Key words irinotecan; CPT-11; SN-38; pharmacokinetics; metabolism

Irinotecan (CPT-11) is a semi-synthetic derivative of camptothecin used for the treatment of cancer. CPT-11 is a prodrug which is hydrolyzed to its active form, SN-38 [(+)-(4S)-4,11-diethyl-4,9-dihydroxy-1H-pyra[3',4':6,7]-indolizino[1,2-b]quinoline-3,14(4H,12H)-dione], which is devoid of the piperidino-piperidino moiety in CPT-11. Animal studies and clinical trials revealed that SN-38 appears in the plasma after dosing with CPT-11, and that the plasma concentration of SN-38 is high enough to exhibit cytotoxic effects.1−4 The conversion occurs in plasma and in the homogenates of tissues, including the liver, kidney and intestine of various species, and the converting enzyme has been identified as carboxylesterase.5 Although SN-38 levels after dosing with CPT-11 in various species is well documented, little is known about the pharmacokinetic profiles of SN-38 after dosing with SN-38 itself.

In this study 14C-labeled SN-38 was intravenously dosed to rats, and plasma levels, tissue distribution and excretion were investigated. The dispositional characteristics of SN-38 were compared with those of CPT-11.

MATERIALS AND METHODS

Drugs and Chemicals 14C-Labeled SN-38 (3.85 MBq/mg) was purchased from Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan); it is synthesized by the hydrolysis of 14C labeled CPT-11 with 1 M NaOH (Fig. 1). Non-labeled SN-38 was supplied from Yakult Honsha Co., Ltd. (Tokyo, Japan). All other reagents were of analytical grade.

Animals Male Wistar rats (221—331 g) were purchased from Charles River Japan, Inc. Animals were housed in a temperature (23 ± 2°C) and humidity (55 ± 15%)-controlled room with 12 h light per day. Mouse/rat diet F-2, in pellet form (Funabashi Farms, Japan) and tap water were allowed ad libitum. To determine biliary excretion, polyethylene tubing (PE 10 Clay Adams, U.S.A.) was inserted into the common bile ducts of the animals under ether anesthesia. After verifying a regular flow of bile, the abdominal incisions were closed and the animals put into restraining cages to collect bile. Food and water was allowed ad libitum during the bile sampling.

Dosing 14C-Labeled and non-labeled SN-38 were accurately weighed and mixed, then dissolved in 0.9% NaCl solution containing 0.1 M NaOH. To this solution, 0.9% NaCl solution containing 0.1 N HCl was added dropwise to adjust the pH to 8.5 (pH meter determination).

![Chemical Structure of SN-38 and CPT-11](image)

Fig. 1. Chemical Structure of SN-38 and CPT-11

The asterisks indicate the 14C-labeled position.

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The dose was set at 6.1 mg/kg, which is equivalent to 10 mg CPT-11/kg on a molar basis. The specific radioactivity in the solutions was 1.51 MBq/ml for the urinary and fecal excretion study, and 2.17 MBq/ml for the plasma and tissue distribution study, biliary excretion study and whole body autoradiography. The solution was intravenously dosed from the tail vein at a volume of 2 ml/kg.

**Blood Levels and Tissue Distribution** At scheduled time points after dosing, animals were dosed with heparin solution (ca. 100 units), and blood was obtained by exsanguination. Then the liver, kidneys, lungs and the upper part of jejunum were excised. An aliquot of the whole blood was used for the measurement of radioactivity. The remaining blood was centrifuged to obtain plasma, and a part of the plasma was passed through an ultrafiltration apparatus (MPS-3, Amicon, Grace Japan, Inc.). The radioactivity in the plasma and the ultrafiltrate were measured and the protein binding ratio was calculated using the following equation:

\[
\text{Protein binding (\%)} = \frac{(1 - \text{ultrafiltrate radioactivity})}{\text{plasma radioactivity}} \times 100
\]

**Urinary and Fecal Excretion** To collect the urine and feces after dosing with SN-38, the animals were individually placed in metabolic cages. Urine was collected 0—2, 2—4, 4—8 and 8—24 h after administration. Thereafter, the urine samples were collected every 24 h up to 168 h after dosing. The total volume of the excreted urine was measured and an aliquot was taken for the radioactivity measurements. Feces were collected at 0—8, 8—24 h and thereafter every 24 h up to 168 h. The samples were stored at −20°C until analysis.

**Biliary Excretion** After rats were placed in restraining cages, bile was collected at 20, 40, 60, 90, 120, 240, 480 min, 24 and 48 h after dosing. The urine and the feces were also collected. The bile samples were subjected to TLC in order to separate radioactive components.

**Determination of Radioactivity** Aliquots of urine and bile were directly, or after dilution, mixed with Aquasol-2 scintillator (DuPont/NEN, U.S.A.) and measured for radioactivity. Feces were homogenized with saline and the total volume measured. An aliquot of the homogenate was solubilized with a tissue solubilizer (Soluene-350, Packard, U.S.A.). After addition of the scintillator (Hionicflour, Packard), radioactivity was measured. As for the tissues and blood, 1 ml of Soluene-350 was added to about 100 mg of each tissue or an aliquot of blood followed by incubation at 55°C overnight. The solution was decolorized by the addition of a toluene solution saturated with benzoic peroxide and incubated at 55°C for several hours. Its radioactivity was measured by a scintillation counter after the addition of the scintillator (Hionicflour).

**TLC** Portions of the plasma, urine and bile samples were subjected to TLC. Because of the low radioactivity in the plasma samples, they were extracted with methanol and concentrated before being applied to the TLC plates. Urine and bile samples were directly, or after dilution, applied to TLC plates. TLC was conducted using precoated Silica gel 60F_254 glass plates (0.25 mm, Merck, Germany) and a solvent system: n-butanol/acetic acid/water (4:1:1 v/v). After radioactive spots on the TLC plates were determined by autoradiograms, they were removed into vials. After the addition of 1 ml of methanol, Aquasol-2 scintillator was added, and the radioactivity was measured with a liquid scintillation counter. To identify the metabolites of SN-38, an aliquot of plasma obtained at 5 min after dosing was incubated with or without β-glucuronidase (1000 unit type IX-A, Sigma Co., U.S.A.) at 37°C for 40 min, and the solutions were subjected to TLC.

**Whole Body Autoradiography** Five minutes, 1 and 24 h after the administration of 14C-SN-38, rats were sacrificed by ether anesthesia. Body hair was removed and the animals frozen in a dry ice–acetone bath. They were then embedded, using 7% carboxymethylcellulose gel, onto a plastic plate, and again frozen in a dry ice–acetone bath. Using cryomicrotome (PMV-450, PMV, Sweden), whole body sections (ca. 30 μm) were prepared and freeze-dried in the cryostat. The freeze-dried sections were covered with thin wrap film (Lumilar, Tohre, Japan) and brought into contact with X-ray film and exposed for 10—30 d in the dark.

**Pharmacokinetic Analysis** The area under the blood concentration–time curve (AUC) and mean residence time (MRT) were calculated using the trapezoidal rule with extrapolation to infinity. Half-life was estimated by the non-linear least squares method using a personal computer. Total body clearance (CLtn) and the volume of distribution at steady state (Vdss) were calculated as follows.

\[
CLtn = \frac{\text{dose}}{AUC}
\]
\[
Vdss = \frac{\text{MRT} \times CLtn}{dose}
\]

**RESULTS**

**Pharmacokinetics** Plasma concentration–time profiles after dosing with 14C-SN-38 are shown in Fig. 2. The radioactivity level in plasma decayed bi-exponentially with a terminal half-life of 9.91 h.

The radioactive components in plasma were separated by TLC. In TLC autoradiograms, three major spots were found within 1 h after dosing (Fig. 3). The spot showing the highest Rf value was identified as SN-38 by chromatography with the authentic sample. The spot

![Fig. 2. Plasma Concentration of Radioactivity (O), SN-38 (●), SN-38 Glucuronide (△) and an Unknown Metabolite (M-2) (□) after a Single i.v. Dosing with 14C-SN-38 to Non-fasted Male Rats (n=3, 6.1 mg/kg)]](image)
closest to the origin was suggested to be SN-38 glucuronide (SN-38 Glu), as has been reported previously. It completely disappeared on TLC after incubation with β-glucuronidase, which supports the above identification (Fig. 4). The spot between these two spots was unidentified and named M-2. It disappeared after a freeze-thaw cycle, thus indicating its instability.

The radioactivity consisted mainly of unchanged SN-38 at the beginning after dosing, but it was gradually replaced with SN-38 glucuronide and M-2. These two metabolites accounted for the plasma radioactivity in the elimination phase. The unchanged SN-38 rapidly decreased, with a half-life of about 7 min, and 2 h after dosing it could not be detected. The pharmacokinetic parameters for SN-38 are summarized in Table 1. Protein binding of radioactivity was relatively high, at 75.9—93.0% (Table 2).

**Tissue Distribution** Figure 5 shows radioactivity in the tissues after i.v. dosing with SN-38. Five minutes after dosing, the radioactivity concentration was 65.87, 39.48, 8.62 and 3.44 μg/g in the kidneys, liver, jejunum and lungs, respectively. Then the concentration was decreased biexponentially, with little radioactivity being found 168 h after dosing.

**Urinary and Fecal Excretion** Figure 6 shows the excretion of radioactivity in the urine and feces. Within 168 h after dosing, 70.0% of the dosed radioactivity was found in the feces and 28.4% in the urine. Thus almost all the dosed radioactivity (98.4%) was recovered in feces and urine within 168 h. The urinary radioactivity consisted mainly of SN-38 (Table 3). The excretion of SN-38 glucuronide in the urine was minor and M-2 was not detected in the urine.

**Biliary Excretion** Figure 7 shows the cumulative ex-

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**Table 1. Pharmacokinetic Parameters for SN-38 after a Single i.v. Dosing of 14C-SN-38 and Parameters for CPT-11 after Dosing of 14C-CPT-11 to Non-fasted Male Rats**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SN-38</th>
<th>CPT-11</th>
</tr>
</thead>
<tbody>
<tr>
<td>$AUC$ (μg eq × h/ml)</td>
<td>0.83</td>
<td>4.56</td>
</tr>
<tr>
<td>$MRT$ (h)</td>
<td>0.23</td>
<td>1.43</td>
</tr>
<tr>
<td>$k_e$ (1/h)</td>
<td>6.45</td>
<td>—</td>
</tr>
<tr>
<td>$T_{1/2}$ (h)</td>
<td>0.11</td>
<td>1.54</td>
</tr>
<tr>
<td>$V_{dss}$ (l/kg)</td>
<td>1.69</td>
<td>3.15</td>
</tr>
<tr>
<td>$CL_{tot}$ (l/h/kg)</td>
<td>7.35</td>
<td>2.22</td>
</tr>
<tr>
<td>$CL_r$ (l/h/kg)</td>
<td>1.61</td>
<td>0.49</td>
</tr>
</tbody>
</table>

a) 6.1 mg/kg (10 mg CPT-11 eq/kg). b) 10 mg CPT-11/kg from ref. 2. $k_e$: elimination rate constant, $V_{dss}$: volume of distribution at steady state, $CL_{tot}$: total body clearance, $CL_r$: renal clearance.

**Table 2. Plasma Protein Binding of Radioactivity after a Single i.v. Dosing of 14C-SN-38 to Non-fasting Male Rats**

| Time (min) | % of binding
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>93.0 ± 0.31</td>
</tr>
<tr>
<td>15</td>
<td>90.1 ± 1.31</td>
</tr>
<tr>
<td>30</td>
<td>79.5 ± 2.85</td>
</tr>
<tr>
<td>60</td>
<td>75.9 ± 2.35</td>
</tr>
</tbody>
</table>

a) Data are expressed as the mean value ± S.E. for three rats.

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**Fig. 4. TLC Autoradiograms after an Incubation of the Plasma with β-Glucuronidase**

1000 units, type IX-A, 37°C for 40 min, lane 2. The control is shown in lane 1. After storage, M-2 (Fig. 3) disappeared.

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**Fig. 5. Tissue Concentration of Radioactivity after a Single i.v. Dosing with 14C-SN-38 at a Dose of 6.1 mg/kg to Non-fasted Male Rats (n = 3)**

Symbols denote (●) plasma, (○) liver, (□) lung, (△) kidney, (△) jejunum.
Fig. 6. Cumulative Urinary and Fecal Excretion of Radioactivity after a Single i.v. Dosing with \(^{14}C\)-SN-38 at a Dose of 6.1 mg/kg to Non-fasted Male Rats (n = 4).
Symbols denote urine (□), feces (○) and total recovery (●).

Table 3. The Components of Radioactivity Excreted in the Urine after a Single i.v. Dosing of \(^{14}C\)-SN-38 to Non-fasted Male Rats*^

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Cumulative excretion (% of dose)</th>
<th>( ^{14}C)-SN-38</th>
<th>SN-38 Glu</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>18.5 ± 2.2</td>
<td>15.5 ± 1.7</td>
<td>2.5 ± 0.4</td>
<td>0.5 ± 0.0</td>
</tr>
<tr>
<td>4</td>
<td>21.4 ± 1.0</td>
<td>17.3 ± 0.9</td>
<td>3.4 ± 0.2</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>8</td>
<td>22.6 ± 1.0</td>
<td>18.2 ± 0.8</td>
<td>3.7 ± 0.2</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>24</td>
<td>24.5 ± 1.1</td>
<td>20.2 ± 0.9</td>
<td>3.7 ± 0.2</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>48</td>
<td>26.2 ± 1.2</td>
<td>21.9 ± 1.1</td>
<td>3.7 ± 0.2</td>
<td>0.6 ± 0.1</td>
</tr>
</tbody>
</table>

*Data are expressed as the mean value ± S.E. for four rats.

Fig. 7. Biliary, Urinary and Fecal Excretion of Radioactivity after a Single i.v. Dosing with \(^{14}C\)-SN-38 to Bile-duct Cannulated Rats (n = 4, 6.1 mg/kg).
Symbols denote bile (▲), urine (□), feces (○) and total recovery (●).

Table 4. The Components of Radioactivity Excreted in the Bile after a Single i.v. Dosing of \(^{14}C\)-SN-38 to Non-fasted Male Rats*^

<table>
<thead>
<tr>
<th>Time</th>
<th>Cumulative excretion (% of dose)</th>
<th>( ^{14}C)-SN-38</th>
<th>SN-38 Glu</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 min</td>
<td>41.7</td>
<td>3.6</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>40 min</td>
<td>49.7</td>
<td>6.3</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>1 h</td>
<td>51.7</td>
<td>7.9</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>1.5 h</td>
<td>52.2</td>
<td>8.6</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>2 h</td>
<td>52.3</td>
<td>9.2</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>4 h</td>
<td>52.5</td>
<td>9.2</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>8 h</td>
<td>52.6</td>
<td>9.2</td>
<td>2.2</td>
<td></td>
</tr>
</tbody>
</table>

*Data are expressed as the mean value for four rats.

The excretion of biliary, fecal and urinary excretion of radioactivity after a single i.v. dose of SN-38 to the rats whose bile-ducts has been cannulated. The radioactivity was passed rapidly into the bile, and accounted for 64.1% of the dosed radioactivity within 48 h. Urinary excretion was (20.3%) and little radioactivity was excreted in the feces (0.1%). The urine could not be collected in two of four bile-duct cannulated rats within 4 h after dosing, probably due to the stress caused by the operation and the restraint by the cages.

The biliary radioactivity consisted mainly of unchanged SN-38 (Table 4). The excretion of SN-38 glucuronide was minor and M-2 could not be found.

**Whole Body Autoradiography** Five minutes after dosing, the highest radioactivity was observed in the intestinal contents, followed by the liver and kidneys (Fig. 8A). The radioactivity in the lungs and skin was at a level very similar to that in the whole blood. Other tissues showed a lower level of radioactivity than the whole blood. The central nervous system showed the lowest radioactivity levels. Most of the glandular tissues such as the salivary glands, pancreas and adrenals, showed low levels of radioactivity, similar to muscle. The radioactivity in the body rapidly decreased, and at 1 h after dosing, except for in the kidney, showed moderate levels (Fig. 8B). Twenty-four hours after dosing, little radioactivity was found in the body except in the kidney and gastrointestinal contents (Fig. 8C).

**DISCUSSION**

The chemical modification of drugs is sometimes employed to improve their physicochemical properties such as stability or water-solubility. CPT-11 is a prodrug that was first developed to improve water-solubility; however, introduction of the piperidino-piperidino moiety may alter the pharmacokinetics of the parent compound, SN-38. Therefore, to estimate the pharmacokinetic effect of this modification, we investigated the pharmacokinetics of SN-38 after dosing with SN-38 itself.

After dosing rats with \(^{14}C\)-labeled SN-38, the radioactivity was mainly excreted into the bile, this being consistent with the results of fecal excretion. Although a small amount of the radioactivity was retained in the body for 168 h after dosing, most of the radioactivity was excreted very rapidly.

Two metabolites (SN-38 Glu, M-2) were found in the plasma. They were the major components of plasma radioactivity at 60 and 120 min after dosing. Their plasma radioactivity at 240 min was too low to determine the levels; however, they seemed to be the major components of plasma radioactivity at that time. A TLC autoradiogram revealed that M-2 disappeared after a freeze-thaw cycle,
suggesting that M-2 is a very unstable conjugate of SN-38. M-2 was not found in the urine, suggesting that M-2 was converted to SN-38 in the kidney. The chemical structure of M-2 could not be determined due to its instability and scarcity. The plasma protein binding was decreased with time, from 93.0 to 75.9%. This decrease might be explained by the replacement of radioactive components in plasma, from SN-38 to its metabolites which have a low affinity to plasma proteins.

As for unchanged SN-38, the plasma half-life was very short, about 7 min. The half-life of SN-38 after dosing with CPT-11 was reported to be much longer, about 2.5 or 2.8 h. Because the cytotoxic effect of SN-38 is time-dependent rather than \textit{AUC}-dependent, long-time exposure of SN-38 is required for cytotoxicity, even when its concentration is low. Therefore, dosing with CPT-11 has an advantage over that with SN-38 in expressing \textit{in vivo} cytotoxic activity. In the pharmacokinetic analysis of SN-38, \( CL_{\text{tot}} \) and \( Vd_{\text{ss}} \) were calculated as 7.35 l·h\(^{-1}\)·kg\(^{-1}\) and 1.69 l·kg\(^{-1}\), respectively. From a previous report on CPT-11 pharmacokinetics,\(^9\) the \( CL_{\text{tot}} \) and \( Vd_{\text{ss}} \) of CPT-11 were calculated as 2.22 l·h\(^{-1}\)·kg\(^{-1}\) and 3.15 l·kg\(^{-1}\), respectively. The larger \( Vd_{\text{ss}} \) and smaller \( CL_{\text{tot}} \) for CPT-11 than for SN-38 lead to a longer half-life for CPT-11. CPT-11 is known to be a major component of the radioactivity in tissues after dosing with CPT-11,\(^8\) suggesting that the sustained release of SN-38 from CPT-11 in tissues can account for a longer plasma half-life of SN-38 after dosing with CPT-11. Lüllmann \textit{et al.} reported the binding of various acidic, neutral and basic
drugs to atrial homogenate of guinea pigs. In this report, acidic drugs showed a higher affinity to human serum albumin (HSA) than to tissue homogenates, whereas basic drugs show little difference in affinity between HSA and tissue homogenates. In the case of neutral drugs that have a high affinity to HSA, such as SN-38, they show a higher affinity to HSA rather than to the tissue homogenate. In the case of CPT-11, the piperidino–piperidino moiety also provides CPT-11 with basic properties, which may contribute to a larger distribution volume than SN-38. The high ratio of protein binding of SN-38 may account for its lower distribution volume. However, the protein binding of CPT-11 could not be determined because of an extensive conversion of CPT-11 to SN-38 by carboxylesterase in plasma, thus preventing a direct comparison of protein binding between CPT-11 and SN-38.

Whole body autoradiograms indicated a low level of radioactivity in glandular tissues, such as adrenal, salivary gland or intestines, whereas a high level of radioactivity was found in these tissues after dosing with 14C-CPT-11. The high concentration in these glandular tissues may have some relationship to the intestinal excretion of CPT-11. After dosing with CPT-11, about 10% of the radioactivity was found in feces, even when the bile ducts of the animals were cannulated. On the other hand, virtually no radioactivity was found in the feces after dosing with SN-38 under the same condition, indicating no direct excretion from the intestine.

In summary, SN-38 showed a smaller distribution volume and a higher clearance than CPT-11, which resulted in a shorter half-life. SN-38 shows no specific distribution to glandular tissues as CPT-11 does. Thus the piperidino–piperidino moiety of CPT-11 is found to affect the disposition of camptothecin derivatives and to increase their water solubility.

REFERENCES