Development of ELISAs for Irinotecan and Its Active Metabolite SN-38

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Two highly sensitive and specific enzyme-linked immunosorbent assays (ELISAs) for the determination of 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carboxyloxy-camptothecin (irinotecan) and 7-ethyl-10-hydroxy-camptothecin (SN-38), an active metabolite of irinotecan, were developed, which are capable of measuring as low as 16 and 160 pg of each drug/ml, respectively.

Anti-irinotecan antibody was obtained by immunizing rabbits with irinotecan conjugated with mercapto-succinyl bovine serum albumin (MS.BSA) using N-(4-diazophenyl)maleimide (DPM) as a heterobifunctional coupling agent. An enzyme marker was similarly prepared by coupling irinotecan with horseradish peroxidase (HRP) via DPM. This ELISA for irinotecan was specific for irinotecan and showed almost no cross-reactivity with its active metabolite SN-38. Anti-SN-38 antibody was obtained by immunizing rabbits with SN-38 conjugated with BSA using the N-succinimidyl ester method. An enzyme marker was prepared by coupling SN-38 with HRP employing DPM. The ELISA for SN-38 was specific to SN-38 and showed a slight cross-reactivity with irinotecan (0.08%). Using the 2 assays, we reconfirmed the rapid metabolism of irinotecan with rat serum. The 2 ELISAs may be a valuable tool for studies of the pharmacokinetics and pharmacodynamics of these drugs.

Key words irinotecan; SN-38; ELISA

Irinotecan, a semisynthetic derivative of the camptothecin, has been shown to be very effective in the treatment of lung, cervical, ovarian, gastric, colorectal, breast, skin cancer and malignant lymphoma. Irinotecan is now widely used in chemotherapy in the treatment of cancer. However, serious side effects such as neutropenia, highly sensitive diarrhea, etc. occur, and the course may be lethal in some cases. In this respect, the usefulness of therapeutic drug monitoring (TDM) is suggested with the purpose of minimizing the toxicity of the drug and of increasing the therapeutic effect. TDM of irinotecan which might serve for cancer chemotherapy requires a simple and sensitive assay method for the quantification of the drug. Irinotecan is mostly metabolized in the human body to an active metabolite SN-38. The cytotoxic activity of SN-38 is approximately 100–500-fold stronger than that of irinotecan, which is considered as a prodrug of SN-38. Therefore, to carry out TDM or a pharmacokinetic study of irinotecan, it is necessary to perform an analytical method specific to both irinotecan and SN-38. Previous pharmacokinetic studies of irinotecan and SN-38 were undertaken using HPLC. ELISA also appears to be an analytical method suitable for this purpose. For most anti-cancer drugs now frequently used in clinical applications, ELISA suitable for TDM or pharmacokinetic studies of these drugs have already been developed. However, an ELISA system for irinotecan or SN-38 has not previously been developed.

We succeeded in producing the first specific antibodies against irinotecan and SN-38. This study describes the methodology for production of the antibodies, the labeling of drugs with horseradish peroxidase (HRP) to act as a tracer, the characterization of the specificity of the antibodies, and the measurement of irinotecan and SN-38 by 2 ELISAs.

MATERIALS AND METHODS

Reagents Irinotecan and SN-38 were supplied from Yakult Honsha Co., Ltd. (Tokyo, Japan). Camptothecin was purchased from Aldrich Chem. Co. (St. Louis, MO, U.S.A.). HRP (for enzyme immunoassay) and 3,3',5,5'-tetramethylbenzidine (TMB) were obtained from Boehringer Mannheim (Mannheim, Germany). Hydrogen peroxide (30%) was purchased from Wako Pure Chemical Industries (Osaka, Japan). N-(4-Aminophenyl)maleimide (APM) was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan).

Preparation of the Immunogen Irinotecan was conjugated to bovine serum albumin (BSA), essentially by the same principle used for the previous preparation of thyrotropin-releasing hormone immunogen (Fig. 1). APM (2 mg, 10.6 μmol) in 40 μl N,N-dimethylformamide (DMF) was acidified by the addition of 100 μl of 1 N acetic acid and then diazotized with sodium nitrite (1.5 mg, 21.7 μmol) in 100 μl distilled water at 0°C for 10 min. Next, 193 μl portions of the above reaction mixture containing N-(4-diazophenyl) maleimide (DPM) (ca. 8.5 μmol) was added directly to irinotecan (5 mg, 8.5 μmol) in a mixed solution comprising 300 μl of DMF and 3 ml of 0.1 M phosphate buffer (pH 7.5), followed by incubation at room temperature for 15 min with vigorous stirring. The resulting irinotecan-DPM was extracted with 2 ml of chloroform and was used without further purification for preparation of the conjugates using mercapto-succinyll BSA (MS.BSA) as the irinotecan immunogen and HRP as the tracer in the ELISA. The yield of irinotecan-DPM was tentatively estimated to be 22.0% according to HPLC measurements of the quantity of non-reacted irinotecan. The molar extinction coefficients of irinotecan-DPM were thus estimated to be 11200 at 280 nm and 22800 at 370 nm, representing those needed to evaluate the quantity of irinotecan conjugated per mole of BSA.

Acetylmercaptosuccinyl BSA (AMS.BSA; 10 mg), containing 17 acetylmercaptosuccinyl groups per BSA molecule, in 0.2 ml of 0.1 M phosphate buffer (pH 7.0) was incubated in 50 μl of freshly prepared 0.5 M hydroxylamine (pH 7.0) at 25°C for 10 min to remove the protecting acetyl group. The resulting MS.BSA, was diluted with 1 ml of 0.1 M phosphate buffer (pH 7.0) containing 3 M urea, added immediately to the irinotecan-DPM in 200 μl of DMF and then incubated at...
25°C for 30 min with vigorous stirring. The reaction mixture was chromatographed on a Sephadex G-100 column (2.8×42 cm) with 0.1 M phosphate buffer (pH 7.0) containing 3 M urea, after which the purified conjugate was examined spectrophotometrically. The latter was estimated to contain approximately 7.1 molecules of irinotecan per BSA molecule, assuming the molar extinction coefficient of BSA to be 43600 at 280 nm and those of irinotecan-DPM to be as described above.

**Preparation of the Immunogen for SN-38**

SN-38-BSA conjugate was prepared by the activated ester method as shown in Fig. 2. A solution of SN-38 (5 mg, 13 μmol) in 300 μl of 0.1 N NaOH was heated at 50°C for 30 min. The resulting SN-38 carboxylate was diluted with 1.7 ml of tetrahydrofuran, mixed immediately with 1-ethyl-3-(3-dimethylaminopropyl)-carbodimide hydrochloride (EDPC) (5 mg, 26 μmol) and N-hydroxysuccinimide (3 mg, 26 μmol), and then stirred at room temperature for 2 h. After removing approximately 1.0 ml of tetrahydrofuran by passing nitrogen through the reaction mixture, the solution (succinimidyl SN-38) was immediately mixed with BSA (10 mg) in 1.0 ml of 0.1 M phosphate buffer (pH 7.0), and incubated overnight at room temperature. The reaction mixture was chromatographed on a Sephadex G-100 column (2.8×42 cm) with an eluant of 0.1 M phosphate buffer (pH 7.0) containing 3 M urea. Then the purified conjugate was examined spectrophotometrically and estimated to contain approximately 1.1 molecules of SN-38 per BSA molecule, assuming the molar extinction coefficients of SN-38 to be 6300 at 280 nm and 23200 at 370 nm, and those of BSA to be 43600 at 280 nm.

**Preparation of Antibody**

All the immunization schedules for the two antigens, irinotecan-BSA and SN-38-BSA, were as follows. One milliliter of a saline of 1 mg of an antigen was emulsified with an equal volume of complete Freund's adjuvant. Each white female rabbit was administered multiple s.c. injections over sites along both sides of their backs. Booster injections were then given 3 times at bi-weekly intervals, using one-half the amount of the dose of the first immunization. The rabbits were bled from an ear vein 10 weeks after immunization began. Fractions of IgG were extracted from the sera with 50% saturated ammonium sulfate and chromatographed on a column of DEAE-Sephadex (2.1×23 cm) using 17.5 mM phosphate buffer (pH 6.8) as an eluant. The fraction passed through the column was lyophilized and used as anti-drug IgG for ELISA.

**Preparation of the Mercaptoacetyl HRP (MS.HRP)**

HRP (2 mg, 50 nmol) in 0.25 ml of 0.1 M phosphate buffer (pH 7.5) was placed with S-acetylmercaptoacetic anhydride (0.9 mg, approximately 5 μmol) in 10 μl of DMF and incubated for 30 min at 30°C with stirring to introduce the thiol groups. Then, 50 μl of 0.1 M Tris-HCl buffer (pH 7.0), 5 μl of 0.1 M EDTA (pH 7.0) and 100 μl of 1 M hydroxyamine (pH 7.0), were added to remove the acetyl groups, and the mixture was incubated for 5 min at 30°C. The reaction mixture was chromatographed on a Sephadex G-25 column (2.0×45 cm) using 0.1 M phosphate buffer (pH 6.0) containing 5 mM EDTA. The average number of thiol groups introduced per HRP molecule was estimated to be 2.2 using 4,4'-dithiodipyridine as described previously. The resulting
MS.HRP was used without further purification for preparation of the conjugates using irinotecan-DPM and SN-38-DPM as the tracer in the ELISA.

Preparation of the Irinotecan-HRP Conjugate

Irinotecan-HRP was prepared by essentially the same principle as the immunogen for irinotecan, using a cross-linker DPM (Fig. 1). In brief, 50 µl of DMF solution containing irinotecan-DPM (approximately 0.4 mg, 0.5 µmol) was mixed with MS.HRP (approximately 0.5 mg, 12.5 nmol) in 2 ml of 0.1 M phosphate buffer (pH 6.0), followed by 30 min incubation at room temperature. The mixture was chromatographed on a Sephadex G-100 column (2.0×40 cm) using 50 mM phosphate buffer (pH 7.4) containing 0.15 M NaCl and 0.1% BSA to remove any remaining small molecular compounds. Fourmillilitre fractions were collected, and fractions 10 to 13, representing the main peak of pure enzyme activity, were chosen as a label in the ELISA. Using the 4,4-dithiopyridine method to determine the thiol group, the conjugate was estimated to contain approximately 2.0 molecules of SN-38 per HRP molecule.

Preparation of the SN-38-HRP Conjugate

SN-38-HRP was prepared by essentially the same principle as the immunogen for irinotecan, using a cross-linker DPM (Fig. 1). APM (2 mg, 10.6 µmol) in 40 µl of DMF was acidified by the addition of 100 µl of 1 N acetic acid and then diazotized with sodium nitrite (1.5 mg, 21.7 µmol) in 100 µl of distilled water at 0°C for 10 min. Next, 30 µl portions of the above reaction mixture containing DPM (ca. 1.3 µmol) was added directly to SN-38 (0.5 mg, 1.3 µmol) in a mixed solution comprising 0.5 ml of DMF and 0.5 ml of 0.1 M phosphate buffer (pH 7.0), followed by incubation at room temperature for 15 min with vigorous stirring. The resulting SN-38-DPM was extracted using 2 ml of chloroform, and this was followed by washing with saturated sodium chloride. After removing chloroform by passing nitrogen through the chloroform layer, the DMF solution was allowed to react with MS.HRP (approximately 0.5 mg, 12.5 nmol) in 2 ml of 0.1 M phosphate buffer (pH 6.0), followed by 30 min incubation at room temperature. The mixture was chromatographed on a Sephadex G-100 column (2.0×40 cm) using 50 mM phosphate buffer (pH 7.4) containing 0.15 M NaCl and 0.1% BSA to remove any remaining small molecular compounds. Fourmillilitre fractions were collected, and fractions 10 to 13, representing the main peak of pure enzyme activity, were chosen as a label in the ELISA. Using the 4,4-dithiopyridine method to determine the thiol group, the conjugate was estimated to contain approximately 2.0 molecules of SN-38 per HRP molecule.

ELISA for Determination of Irinotecan and SN-38

ELISA is based on the principle of competition between enzyme-labeled and unlabeled drugs for an immobilized antibody, followed by measurement of the marker enzyme activity of the immunocomplex bound to the solid phase. Briefly, the well in microtiter plates (Nunc F Immunoplates I; Nunc, Reskiilde, Denmark) were coated by loading 150 µl of anti-irinotecan IgG (20 µg/ml) or anti-SN-38 IgG (20 µl/ml) in 10 mM Tris–HCl buffer (pH 8.5) containing 10 mM NaCl and 10 mM Na2S, and allowed to stand overnight at 4°C. After the plates had been washed twice with 50 mM phosphate buffer (pH 7.4) containing 0.15 M NaCl, 0.1% BSA and 0.05% Tween (buffer A), they were incubated with 200 µl of 50 µM phosphate buffer (pH 7.4) containing 0.15 M NaCl and 2% BSA for 20 min at 37°C to prevent nonspecific adsorption. The anti-Drug IgG-coated wells were then filled with 50 µl of either drug-treated samples, or PBS as a control, followed immediately by 50 µl of the pooled drug-HRP conjugate (diluted 1:1000 and 1:50 in buffer A for irinotecan and SN-38, respectively). The wells were then incubated overnight at 4°C and once again washed briskly with buffer A.

The activity of the enzyme conjugate bound to each well was then measured by the addition of 150 µl of 0.42 mM TMB in 0.05 M acetate–citric acid buffer (pH 5.5) containing 3% dimethyl sulfoxide and 0.01% hydrogen peroxide, followed by incubation of the wells at 37°C for a suitable period. The enzyme reaction was stopped by the addition of 50 µl of 2.0 M H2SO4 to each well, and the resulting color intensity was measured spectrophotometrically at 450 nm using an ELISA analyzer (Molecular Devices; California, U.S.A.).

RESULTS

ELISA The optimal quantities and optimal incubation time for each reaction were established. The dose-response standard curves of irinotecan and SN-38 obtained in the buffer system are shown in Fig. 3. The limits of irinotecan detection by ELISA for were between 3.2 pg and 10 ng/ml of irinotecan. For practical purposes, the working range was ar-
arbitrarily set between 16 and 400 pg/ml based on the precision and accuracy findings for the ELISA (Table 1), which reveals this developed ELISA to be a reproducible technique. Recoveries of 4 different levels of irinotecan ranging from 16 pg to 2 ng/ml were satisfactory, 94.5 to 105.0% (n = 5). The coefficients of variation (c.v.) for intra- and interassays between irinotecan concentrations of 16 pg to 2 ng/ml at 4 different levels each were 2.0 to 23.1% and 5.3 to 19.8% (n = 5 for each), respectively. Detection of the drug in serum samples (50 μl) was also carried out by adding known concentrations. The standard curve yielded was essentially the same as that in the buffer system.

The limits of SN-38 detection by ELISA were between 32 pg and 20 ng/ml of SN-38. For practical purposes, the working range was arbitrarily set between 160 pg and 4 ng/ml based on the precision and accuracy findings for the ELISA (Table 2), which reveals this developed ELISA to be a reproducible technique. Recoveries of 3 different levels of SN-38 ranging from 160 pg to 4 ng/ml at 3 different levels each were 1.9 to 14.1% and 2.0 to 8.3%, respectively. Standard curves were also constructed with various volumes of normal pooled drug-free serum added (0.05 to 50 μl). The standard curves using more than 5 μl of serum differed in shape from that in the buffer system. The limits of drug detection by the ELISA were between 800 pg and 20 ng/ml of SN-38 (data not shown). However, less than 5 μl of serum produced no observable effect.

Specificity The antibody specificity was determined by the displacement of the bound HRP labeled drug by other similar compounds. Values of the cross-reactivity were defined as the ratio of each compound to irinotecan or SN-38 in the concentrations required for 50% inhibition of HRP labeled drug binding to the antibody. The anti-irinotecan antibody showed 0.001% cross-reaction with SN-38, 0.004% with camptothecin and 0.02% with 4-piperidinopiperidine (Table 3). The anti-SN-38 antibody showed 0.08% cross-reaction with irinotecan, 0.07% with camptothecin and 0.002% with 4-piperidinopiperidine (Table 3).

**Table 1. Precision and Accuracy of ELISA for Irinotecan**

<table>
<thead>
<tr>
<th>Added (pg/ml)</th>
<th>Estimated (pg/ml)</th>
<th>Recovery (%)</th>
<th>C.V. (%)</th>
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<tbody>
<tr>
<td>Intraassay</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.0</td>
<td>15.9 ± 1.9</td>
<td>99.4</td>
<td>11.9</td>
</tr>
<tr>
<td>80.0</td>
<td>81.2 ± 1.6</td>
<td>101.5</td>
<td>2.0</td>
</tr>
<tr>
<td>400.0</td>
<td>396.0 ± 35.1</td>
<td>99.0</td>
<td>8.9</td>
</tr>
<tr>
<td>2000.0</td>
<td>2100.0 ± 484.8</td>
<td>105.0</td>
<td>23.1</td>
</tr>
<tr>
<td>Interassay</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.0</td>
<td>16.4 ± 1.7</td>
<td>102.5</td>
<td>10.4</td>
</tr>
<tr>
<td>80.0</td>
<td>78.4 ± 4.8</td>
<td>99.3</td>
<td>6.1</td>
</tr>
<tr>
<td>400.0</td>
<td>408.2 ± 21.6</td>
<td>102.1</td>
<td>5.3</td>
</tr>
<tr>
<td>2000.0</td>
<td>1890.0 ± 374.8</td>
<td>94.5</td>
<td>19.8</td>
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</table>

Values represent the mean ± S.D. of a total of 5 experiments.

**Table 2. Precision and Accuracy of ELISA for SN-38**

<table>
<thead>
<tr>
<th>Added (pg/ml)</th>
<th>Estimated (pg/ml)</th>
<th>Recovery (%)</th>
<th>C.V. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraassay</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>160.0</td>
<td>155.0 ± 21.8</td>
<td>96.9</td>
<td>14.1</td>
</tr>
<tr>
<td>800.0</td>
<td>814.0 ± 15.2</td>
<td>101.8</td>
<td>1.9</td>
</tr>
<tr>
<td>4000.0</td>
<td>4020.0 ± 258.8</td>
<td>100.5</td>
<td>6.4</td>
</tr>
<tr>
<td>Interassay</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>160.0</td>
<td>163.0 ± 13.5</td>
<td>101.9</td>
<td>8.3</td>
</tr>
<tr>
<td>800.0</td>
<td>796.8 ± 16.2</td>
<td>99.6</td>
<td>2.0</td>
</tr>
<tr>
<td>4000.0</td>
<td>3944.0 ± 196.7</td>
<td>98.6</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Values represent the mean ± S.D. of a total of 5 experiments.

**Measurement of Irinotecan and SN-38 in Rat Serum**

Irinotecan is known to be metabolized by carboxylesterase in rat serum to SN-38. Initial applications of the 2 ELISAs were made to measure irinotecan and SN-38 concentrations following addition of irinotecan into rat serum. In brief, irinotecan at 1 μg/ml was incubated with rat serum for various periods at 37°C. The serum was immediately diluted 10-fold with buffer A containing 1 mm disopropylfluorophosphate (DFP), an inhibitor of serine esterases such as carboxylesterases and cholinesterases, to prevent the further metabolism of irinotecan to SN-38, and the solution was stored at −20°C until assayed for drug concentration. The solution was diluted with buffer A containing 1 mm DFP to obtain irinotecan and SN-38 concentrations appropriate for measurement by the 2 ELISAs. As shown in Fig. 4, immediately after the start of the reaction, a rapid decrease in irinotecan and increase in the reaction to generate SN-38 were observed. However, from 5 min after the initiation of the reaction, the reaction tended to decrease. At 15 min from the initiation of reaction and thereafter, the reaction proceeded at a constant rate. Then, the number of mols of the decreased irinotecan approximately agreed with the number of mols of the generated SN-38.

**DISCUSSION**

Two specific and sensitive ELISAs for irinotecan and its active metabolite SN-38, which are suitable for TDM and pharmacokinetic studies on these drugs, have been developed. To our knowledge, there is no previous study demonstrating specific antibody production against irinotecan or SN-38. One probable reason is that these drugs have no suitable reactive structure for making immunogen such as the compound-BSA conjugate.
Table 3. Specificity of Anti-Irinotecan IgG and Anti-SN-38 IgG

<table>
<thead>
<tr>
<th>Compounds</th>
<th>% Cross-reaction (50%)</th>
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<tbody>
<tr>
<td></td>
<td>Anti-Irinotecan IgG</td>
</tr>
<tr>
<td>Irinotecan</td>
<td>100</td>
</tr>
<tr>
<td>SN-38</td>
<td>0.001</td>
</tr>
<tr>
<td>Camptothecin</td>
<td>0.004</td>
</tr>
<tr>
<td>4-Piperidinopiperidine</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Irinotecan immunogen and irinotecan-HRP conjugate (as a tracer) were prepared using DPM with 2 selective, functional diazo and maleimide groups as a cross-linking agent. In the present study, the site of azo-coupling of irinotecan by DPM was not strictly identified, but was predicted to be at the C-9 or C-11 position of the irinotecan molecule, judging from the typical azo-coupling reaction of compounds possessing a phenol moiety. The resulting irinotecan-DPM was conjugated by thiolation with the thiol groups of MS.BSA (immunogen) and MS.HRP (enzyme marker). The irinotecan-DPM was found to be very stable in anhydrous ethyl acetate and can be stored for >10 months at −20°C, during which time the maleimide group is fully maintained. Therefore, a major advantage of using irinotecan-DPM is that it can easily be used for conjugation with proteins containing thiol groups whenever such conjugates are needed. The present conjugation method is simple, mild and reproducible and does not result in extensive (intra- or intermolecular) self-coupling of irinotecan or proteins, and protein conjugates thus prepared can easily and rapidly be purified by gel filtration.

At first, using a method similar to that of irinotecan, SN-38-antigen and SN-38-HRP were prepared, and ELISA for SN-38 was developed. However, the newly developed ELISA for SN-38 exhibited strong cross-reaction to irinotecan and it was not specific to SN-38. Therefore, the lactone ring of SN-38 was ruptured, and the resulting carboxyl group was changed to active ester using the N-hydroxysuccinimide method and was bound to BSA, and the SN-38 immunogen was prepared (Fig. 2). However, we attempted to prepare SN-38-HRP conjugate (as a tracer) using the same N-hydroxysuccinimide method as in the procedure to prepare SN-38 immunogen, but we did not succeed in preparing the enzyme conjugate with a high enzyme activity and immune activity. This may be because a direct reaction with the enzyme was performed without separately purifying the SN-38 active ester, and that polymerized side-products of the enzyme and SN-38 were produced, and this may have resulted in the decreased enzyme activity and immune activity. Therefore, SN-38-HRP was prepared by essentially the same procedure as the irinotecan-HRP, using a cross-linker DPM.

The ELISA for irinotecan can detect as little as 16 pg/ml of irinotecan and is reproducible, as indicated by the overall mean c.v. of 10.9% intraassays and interassays at 4 different levels of irinotecan (Fig. 3 and Table 1). The standard curve in the serum system was similar to that in the buffer system. The ELISA for SN-38 can detect as little as 160 pg/ml of SN-38 and is reproducible, as indicated by the overall mean c.v. of 6.3% intraassays and interassays at 3 different levels of SN-38 (Fig. 3 and Table 2). In the measurement in serum, there were some influences, and the sensitivity was approximately 5-fold lower. However, no influence was found when the serum was diluted greater than 10-fold.
Previous pharmacokinetic studies of irinotecan and SN-38 have been undertaken using HPLC by fluorescence detector. The limits of quantitation for irinotecan and SN-38 were 30 and 1 ng/ml, respectively. This previous HPLC appears to be 2000 and 6-fold less sensitive than the present ELISA for irinotecan and that for SN-38, respectively.

A site heterologous enzyme labeling method often shows high sensitivity compared with an homologous enzyme labeling method. However, the sensitivity of ELISA for SN-38 (site heterologous enzyme labeling method) was 10-fold less sensitive than that of ELISA for irinotecan (homologous enzyme labeling method). These findings suggest that the differences in sensitivity might be due to the differences in the affinity to the antibody.

The specificity of ELISA for irinotecan and SN-38 are shown in Table 3. The anti-irinotecan antibody specificity was directed mainly toward irinotecan, although there were negligible cross-reactivities with the major metabolites of irinotecan (SN-38) and irinotecan analogue (camptothecin). These findings suggest that anti-irinotecan antibody precisely recognizes the 4-piperidino-piperidine site and other sites in the vicinity which are structural sites specific to irinotecan. In concentration transition in human blood, SN-38 shows a concentration of more than several percent of that of irinotecan. In this respect, high specificity is required in cases of ELISA for SN-38. As shown in Table 3, the anti-SN-38 antibody showed a very slight cross-reactivity with irinotecan (0.08%). This ELISA for SN-38 may show enough specificity to quantify SN-38 for pharmacokinetic studies in human. In addition, the anti-SN-38 antibody showed a very slight cross-reactivity with camptothecin (0.07%) which lack an hydroxyl group at the C-10 position and an ethyl group at the C-7 position in SN-38. These findings indicate that the antibody can precisely recognize the hydroxyl group at the C-10 position in SN-38.

As a demonstration of the potential of the 2 ELISAs, a preliminary metabolic study of irinotecan in rat serum was performed (Fig. 4). In rat serum, a rapid metabolic change from irinotecan to SN-38 was detected. Also, there was an approximate agreement between the number of mols of the generated SN-38. These findings suggest that the concentration of irinotecan and SN-38 after administration of irinotecan can be specifically determined using these 2 ELISAs.

Irinotecan and SN-38 can undergo a reversible, pH-dependent hydrolysis in which the closed lactone forms are converted to the open carboxylate forms and vice versa. Recently, it was reported that it was also important to determine the concentrations of these lactone and carboxylate forms in plasma to understand the antitumor activity of irinotecan applications. However, it has not yet been confirmed whether anti-irinotecan antibody exhibits different cross-reactivity to lactone and carboxylate forms of irinotecan or whether anti-SN-38 antibody shows different cross-reactivity to lactone and carboxylate forms of SN-38. This matter must now be elucidated by further studies of the specificity of these antibodies. However, the 2 ELISAs presented here are sensitive, specific, reproducible and adaptable enough for analyses of a large number of samples. The assays should be a valuable tool in studies of the TDM and pharmacokinetics of irinotecan and SN-38.

REFERENCES