— Regular Article —

Feasibility of Bioavailability Testing by Simultaneous Determination of Serum Concentrations of Tegafur and 5-fluorouracil after TS-1 Oral or Tube Administration for Chemotherapy in Oral Cancer Patients

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TS-1 is an oral anticancer agent comprising three components: two biochemical modulators of 5-fluorouracil (5-FU) and tegafur (FT), a prodrug of 5-FU. TS-1 displays potent anti-tumor activity by maintaining effective 5-FU concentrations in serum for a prolonged period. FT is gradually converted to 5-FU *in vivo* via 5'-hydroxylation mediated by cytochrome P450s. As a result, genetic polymorphisms can cause wide individual differences in serum concentration of 5-FU after administration of TS-1, requiring monitoring of serum concentration of 5-FU for each patient. Chemotherapy with TS-1 plays a major role in the treatment of oral cancer. In cases where a patient with oral cancer shows dysphagia, TS-1 may need to be tube-administered. Although tube administration by the simple suspension method has been recommended from a biosafety perspective, particularly for anti-cancer agents, its efficacy remains unclear. We established a simple high-performance liquid chromatography method for simultaneous determination of FT and 5-FU levels at clinical sites by modifying a method reported in the literature.[1)] Unlike the original method, this simplified method does not require extraction procedures to separate FT and 5-FU, and requires only 250 μl of serum. Using this method, we compared FT concentrations in the sera of oral cancer patients administered TS-1 orally or via a tube-assisted simple suspension method. No significant differences in FT concentrations were apparent between these two modes of administration. TS-1 treatment by tube administration using the simple suspension method thus seems useful for patients with dysphagia as an alternative to oral dosage.

Key words —— TS-1, serum concentration, oral cancer, simple suspension method

INTRODUCTION

Standard treatments for oral cancer include surgery, chemotherapy, radiotherapy or a combination of these treatments. As a chemotherapeutic agent, 5-fluorouracil (5-FU) has been playing a central role pre- and/or postoperatively.[2)] TS-1 is an oral anti-tumor agent consisting of three active ingredients: tegafur (FT), gimeracil and potassium oxonate. FT is a prodrug of 5-FU, gimeracil is a dihydropyrimidine dehydrogenase inhibitor and potassium oxonate attenuates the gastrointestinal toxicity of FT.[3)] The chemical structures of 5-FU and FT are depicted in Fig. 1. TS-1 has been reported to be equally effective or superior to 5-FU,[4–6)] producing excellent results in the treatment of oral cancer.[7–9)]

The pharmacokinetics of 5-FU after TS-1 oral administration reportedly vary from person to person and toxicity appears closely related to pharmacokinetic parameters.[10–13)] Kuwahara *et al.* (2009)

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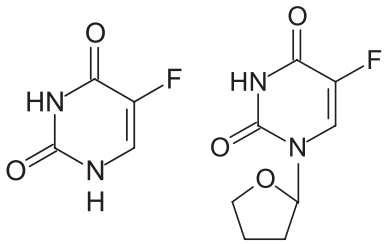


5-FU FT

Fig. 1. Chemical Structures of FT and 5-FU

tried to decrease the incidence of toxicity by adjusting the dose of 5-FU in 5-FU/cisplatin-based chemoradiotherapy.[10] Minimizing the side effects of anti-cancer chemotherapeutics might be clinically beneficial if serum concentrations can be readily determined at clinical sites. High-performance liquid chromatography (HPLC) is the method of choice for this purpose. The present study first confirmed simultaneous determination of FT and 5-FU levels in low-volume serum samples after oral administration of TS-1 by applying the HPLC method developed by Chu *et al*. (2003).[1]

Chemotherapy with TS-1 has been widely employed in the treatment of oral cancer. In cases where oral cancer patients suffer from dysphagia (or other swallowing disorders), TS-1 may have to be tube-administered by opening capsules instead of oral administration. Recently, treatment of such patients using the simple suspension method has been recommended,[14, 15] with drugs delivered via a tube as a suspension after spontaneous decay by soaking in hot water at 55°C for 10 min.[16, 17] The simple suspension method can avoid problems associated with the process of crushing tablets and removing capsules, such as the time-intensive nature of dispensing, loss of applied dose, occlusion of the tube and occupational exposure. If this new method of administration is to be used clinically with confidence, however, a lack of significant differences between the conventional and novel methods must first be confirmed. To achieve this goal, serum concentrations of FT and 5-FU were measured by HPLC after TS-1 was administered orally or by a tube-assisted simple suspension method and pharmacokinetic parameters of FT were compared.

MATERIALS AND METHODS

The clinical part of this study was performed at Ichikawa General Hospital, Tokyo Dental College and the analytical part was mainly undertaken at the Faculty of Pharmaceutical Sciences, Toho University.

Anticancer Drugs and Chemicals —— TS-1 capsules are produced by Taiho Pharmaceutical (Tokyo, Japan). FT, 5-FU, 5-bromouracil (5-BU) and acetic acid were purchased from Sigma Aldrich (Tokyo, Japan). Ammonium sulfate, ethyl acetate, HPLC-grade isopropanol and methanol were obtained from Wako Pure Chemical Industries (Osaka, Japan). The water used throughout this study was purified using Milli-Q Labo equipment (Nihon Millipore, Tokyo, Japan).

Patients —— All patients were required to score ≤ 2 on a scale between 0 and 4 according to the performance status (PS) classification of the World Health Organization (WHO) and to have adequate hematopoietic function.

Six patients with histologically identified oral cancer were enrolled in studies of serum levels of FT and 5-FU over time after oral administration of TS-1 (3 men, 3 women; median age, 64 years; range, 43–76 years).

Bioequivalence studies between oral administration and tube administration by simple suspension method investigated seven patients [5 men (71%), 2 women (29%); mean age, 63 years; range, 43–72 years] with oral administration and four patients [3 men (75%), 1 woman (25%); mean age, 63 years; range, 57–69 years] with tube administration. Both groups were thus fairly well-matched in terms of age and sex distributions.

Ethical clearance approval (no. 20–1, 137) was granted by the Ethics Committees of Tokyo Dental College and the Faculty of Pharmaceutical Sciences at Toho University. Informed consent was obtained from all patients prior to data collection in accordance with the Declaration of Helsinki.

Administration and Blood Sampling —— Patients received TS-1 twice a day for 2 weeks (days 1–14), with doses determined based on body surface area (BSA) in the clinical setting: BSA $< 1.25\,m^2$, 80 mg/body per day; $1.25\,m^2 \leq$ BSA $< 1.5\,m^2$, 100 mg/body per day. Blood samples were drawn on day 8.

To determine the time course of serum FT and 5-FU concentrations after oral administration of TS-1, blood samples were drawn at 0, 1, 3, 5, 7, 9 and 11 hr after TS-1 administration. For the comparison of pharmacokinetics of FT between oral administration and tube administration using the single sus-

pension method, samplings were carried out at 0, 1, 3 and 12 hr after TS-1 administration.

Blood samples were centrifuged for 5 min at 3000 rpm and serum samples were stored at −80°C until HPLC analysis.

Determination of Serum Concentrations of FT and 5-FU —— The HPLC assay method reported by Chu *et al.* (2003) for 500 µl of dog samples was applied to half volumes of human samples.

A mixture of serum sample (250 µl), methanol (50 µl) and 10 mg/l aqueous solution of 5-BU (30 µl) as internal standard (IS) was placed in a centrifuge tube, and then 50 mg of ammonium sulfate was added. The whole mixture was vortex-mixed for 1 min and centrifuged for 5 min at 6000 × *g*. Aliquots (1 ml) of a solvent mixture [isopropanol · ethyl acetate, 15 : 85 (v/v)] were added to the supernatant tubes, which were then vortex-mixed again and centrifuged for 15 min at 6000 × *g*. The organic layer was transferred to another tube and evaporated to dryness at 40°C under nitrogen stream. Residues were dissolved in a mobile phase (75 µl) and 20 µl of the solution was injected into the HPLC system.

The HPLC system consisted of an LC-10AD VP pump (Shimadzu, Kyoto, Japan) equipped with a Shimadzu SPD-10A VP UV detector operated at 260 nm and a Spherisorb ODS2 analytical column (250 × 4.6 mm, 5 µm internal diameter; Waters, Tokyo, Japan).

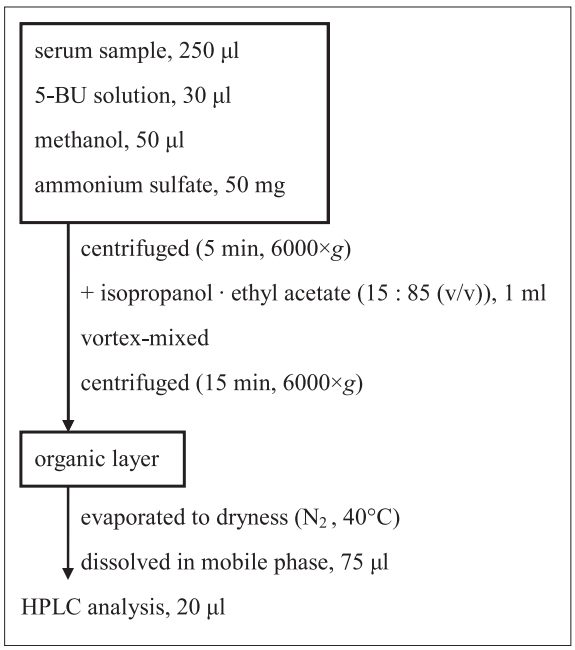
The column was maintained at room temperature (22°C) and eluted with the mobile phase [10 mM acetic acid · methanol, 90 : 10 (v/v)] maintained at a flow rate of 1.0 ml/min.

The analytical procedure for determining FT and 5-FU is summarized in Scheme 1.

Pharmacokinetic Parameters —— The area under the concentration-time curve [AUC (ng·hr/ml)] was calculated according to the trapezoidal rule, using data obtained at 0, 1, 3, 5, 7, 9 and 11 hr after administration of TS-1 on day 8.

Data were processed statistically using Student's *t*-test and Fisher's exact test. Values of $p < 0.05$ were considered significant and results are expressed as mean with standard deviation (SD). Pharmacokinetic parameters were analyzed for bioequivalence parametrically after logarithmic transformation. Bioequivalence was accepted if the 90% confidence interval (CI) for the ratio between test and reference was 0.80–1.25.

Scheme 1. Analytical Procedure for Assay of FT and 5-FU



RESULTS AND DISCUSSION

The anti-cancer agent TS-1 contains FT and has been widely used as a chemotherapy for oral cancer. As FT is gradually converted into the active form of 5-FU in the liver by cytochrome P450s, genetic polymorphisms are responsible for wide individual differences in serum concentrations of 5-FU after administration of TS-1.

Exposure to 5-FU is clearly correlated with both toxicity and efficacy. As TS-1 has many serious adverse effects, monitoring of FT and 5-FU is considered necessary to achieve optimal outcomes when using TS-1 for chemotherapy in clinical practice.

HPLC and gas chromatography-mass spectrometry have been widely used for determining levels of FT and 5-FU.[18,19] Several analytical methods have been reported for assaying FT and 5-FU in biological fluids, such as gas chromatography-negative ion chemical ionization mass spectrometry[20] and liquid chromatography-mass/mass spectrometry (LC-MS/MS).[21] However, these methods are unsuitable for routine clinical use, as they include many complicated steps and require highly sophisticated equipment. The analytical method used in this investigation was established by modifying the HPLC procedure reported by Chu *et al.*,[1] which was originally developed for simultaneous determination of 5-FU and FT levels in samples of dog plasma. Assay procedures for FT and 5-FU adopted in this study are summarized in Scheme 1.

Table 1. Pharmacokinetic Parameters of FT and 5-FU after Administration of TS-1

Consecutive administration of TS-1		C_{max} (ng/ml)	T_{max} (hr)	AUC (ng·hr/ml)	$T_{1/2}$ (hr)
50 mg (n = 3)	FT	1476.0 ± 488.4	1.0 ± 0	7830.9 ± 2449.1	8.6 ± 1.3
	5-FU	252.1 ± 62.2	1.7 ± 1.2	861.3 ± 293.0	3.4 ± 1.1
40 mg (n = 3)	FT	980.4 ± 371.2	1.7 ± 1.2	6291.2 ± 1987.2	10.0 ± 3.0
	5-FU	205.2 ± 99.6	1.7 ± 1.2	978.8 ± 364.5	2.5 ± 1.0

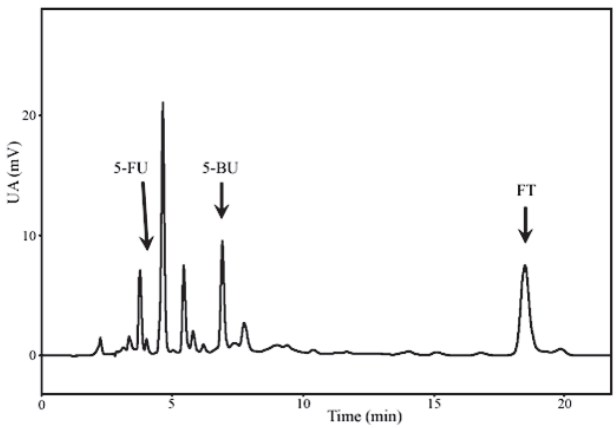


Fig. 2. Chromatogram of Human Serum Sample after Oral Administration of TS-1

Chromatographic conditions: pump, LC-10AD (Shimadzu); column, C18 Spherisorb ODS2 (250 mm × 4.6 mm, 5 μm internal diameter; Waters); mobile phase, 10 mM acetic acid · methanol, 90 : 10 (v/v); flow rate, 1.0 ml/min; detector wavelength, 260 nm; column temperature, room temperature (set at 22°C); retention time, [5-FU : 4.0 min, FT : 18.2 min, 5-BU (IS) : 6.8 min].

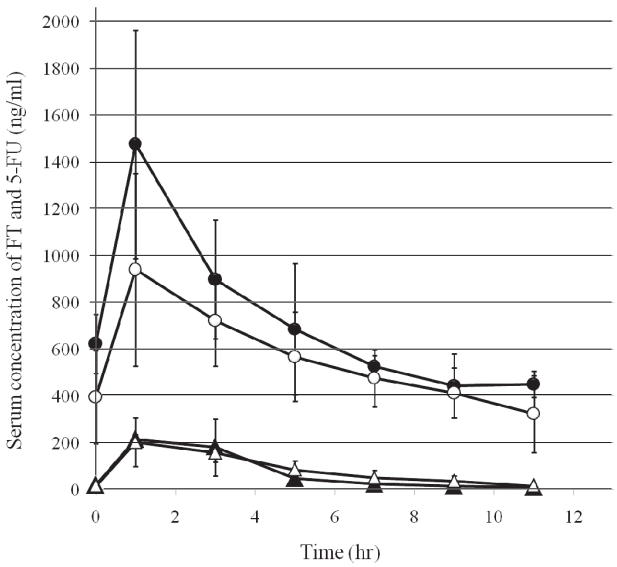


Fig. 3. Serum Concentrations of FT and 5-FU after Oral Administration of TS-1

Serum concentrations of FT (circle) and 5-FU (triangle) were monitored on day 8 after administration of either 50 mg (closed) or 40 mg (open) of TS-1. ●: FT (50 mg TS-1), ▲: 5-FU (50 mg TS-1), ○: FT (40 mg TS-1), △: 5-FU (40 mg TS-1). Each point and bar represent mean ± S.D. (50 mg, n = 3; 40 mg, n = 3).

The modified method was rapid and simple enough for routine clinical assay and enabled simultaneous measurement of FT and 5-FU concentrations using small volumes of human serum (250 μl).

A representative chromatogram from a human serum sample after oral administration of TS-1 (50 mg) is shown in Fig. 2. Both peaks of 5-FU (4.0 min) and FT (18.2 min) were clearly separated from that of the 5-BU internal standard (IS, 6.8 min). Calibration curves for FT and 5-FU were linear up to 3000 ng/ml (r^2 > 0.990, n = 5) and 1200 ng/ml (r^2 > 0.989, n = 5), respectively. The detection limit was 4 ng/ml [Signal/Noise (S/N) = 3].

Serum concentrations of FT and 5-FU monitored on day 8 after administration of a typical clinical dose of either 40 or 50 mg of TS-1 (depending on BSA of the patient) are shown in Fig. 3. Pharmacokinetic parameters calculated from these data are shown in Table 1. Serum concentrations of both FT and 5-FU peaked 1–3 hr after oral administration, then decreased gradually (Fig. 3).

A higher FT value does not necessarily mean higher 5-FU concentration, reflecting individual metabolic variability. Furthermore, only 250 μl of serum sample was found to be sufficient for simultaneous determinations of FT and 5-FU.

Bioequivalence studies were conducted with the serum after oral or tube administration of TS-1 using the simple suspension method. FT concentrations in serum after oral or tube administration of TS-1 were measured to evaluate the efficacy of TS-1 administered using the simple suspension method (Fig. 4). Mean serum levels of FT at 0, 1, 3 and 12 hr after oral (tube) administration of 50 mg TS-1 were 680.8 ± 236.2 ng/ml (634.3 ± 109.9 ng/ml), 1441.7 ± 499.9 ng/ml (1418.1 ± 222.5 ng/ml), 1264.8 ± 431.0 ng/ml (1212.0 ± 157.0 ng/ml) and 780.6 ± 306.2 ng/ml (758.5 ± 132.1 ng/ml), respectively. Pharmacokinetic parameters, C_{max} and AUC, of FT after the oral (tube) administration were 1606.8 ± 359.3 ng/ml (1314.8 ± 232.0 ng/ml) and 12835.7 ± 3562.6 ng·hr/ml (12070.0 ± 1103.1 ng·hr/ml), respectively (Table 2). The values of C_{max} and AUC for 5-FU after the oral (tube) administration were 209.1 ± 73.3 ng/ml (152.4 ± 80.5 ng/ml), 1298.9 ± 510.2 ng·hr/ml (1013.9 ± 456.2

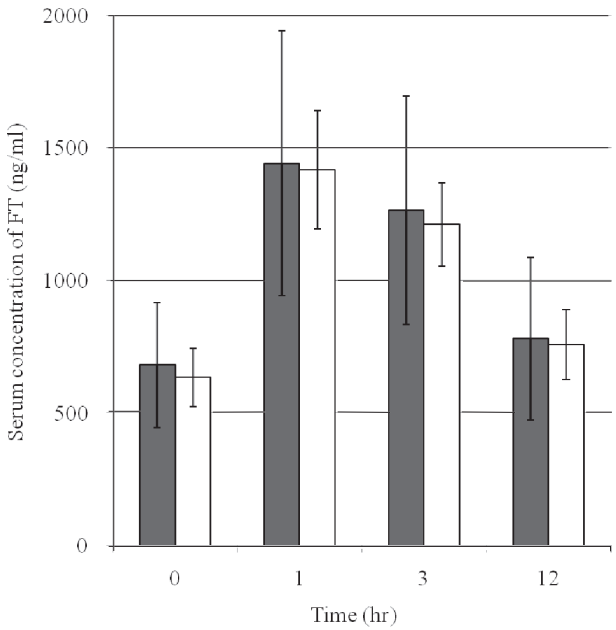


Fig. 4. Serum Concentrations of FT over Time after Oral- or Tube-administered TS-1 Using the Simple Suspension Method

Serum concentrations of FT were monitored on day 8 after administration of 50 mg TS-1 (■: oral administration, □: tube administration using simple suspension method). Each point and bar represent mean ± S.D. (oral, $n = 7$; simple suspension method, $n = 4$). Patient demographics were similar in both groups.

Table 2. Pharmacokinetic Parameters for Oral and Simple Suspension Method from Bioequivalence Tests

Pharmacokinetic parameters	C_{max} (ng/ml)	AUC (ng·hr/ml)
Oral	1606.8 ± 359.3	12835.7 ± 3562.6
Simple suspension method	1314.8 ± 232.0	12070.0 ± 1103.1
Simple suspension method /Oral	0.97	1.01
90% CI of ratio	0.86–1.09	0.87–1.15

Pharmacokinetic parameters were tested for bioequivalence parametrically after logarithmic transformation.

ng·hr/ml), respectively. Thus, serum concentration of 5-FU differed from patient to patient, reflecting individual metabolic variabilities, these methods were comparatively evaluated by the serum concentration of FT. Oral administration and tube administration using the simple suspension method were found to be bioequivalent, as 90% confidence intervals of the ratios for C_{max} and AUC between these two methods were within the range of 0.80–1.25 (Table 2).

In the previous report, we investigated the frequency of adverse experiences associated with administration method, oral administration or tube administration using the simple suspension method.[14] The adverse experiences including, leucopenia, neutropenia, anorexia, diarrhea, stomatitis and hand-foot syndrome were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3. In conclusion, there was no significant difference between these two methods in the frequency of adverse experiences.

Generally speaking, oral drugs enable patients to receive medication on an outpatient basis, which helps patients to maintain quality of life. Patients with oral cancer often suffer from dysphagia, complicating oral administration. As a result, patients have often resorted to tube administration by crushing tablets or opening capsules to allow them to take their medication. However, crushing tablets and removing capsules have various disadvantages, such as the greatly increased time required for dispensing, loss of applied dose, occlusion of the tube and occupational exposure. The finding that the simple suspension method, which is free of these problems, proved to be as effective as oral administration in the TS-1 treatment of oral cancer is thus encouraging. TS-1 is currently in wide use in Japan for the treatment of not only oral cancer, but also cancers such as gastric, colorectal, breast and non-small cell lung cancers. Treatment by tube administration using the simple suspension method has thus been highlighted for patients with dysphagia.

Conversely, attention must be given to individual differences in serum concentrations of 5-FU when TS-1 is administered. In some cases, chemotherapy must be suspended due to serious adverse effects even if curative effects are provided.

When side effects are not so severe, chemotherapy can be continued by adjusting the administration dose of TS-1. Our expectation is that the proposed analytical method can be utilized for simultaneous monitoring serum levels of FT and 5-FU, improving safety and optimizing treatment with TS-1.

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