Acta Pharmacologica Sinica (2013) 34: 570–580
 1671-4083/13 $32.00
www.nature.com/aps

Original Article

Pharmacokinetic evaluation of novel oral fluorouracil antitumor drug S-1 in Chinese cancer patients

Zhi-xiang ZHUANG[#], Hong ZHU[#, *], Ji WANG, Min-gao ZHU, Hui WANG, Wang-yang PU, Hua-hui BIAN, Lei CHEN, Hong ZHANG

Department of Oncology, the Second Affiliated Hospital of Soochow University, Suzhou 215004, China

Aim: S-1 is an oral anticancer fluoropyrimidine formulation consisting of tegafur, 5-chloro-2,4-dihydroxypyridine and potassium oxonate. The aim of this study was to evaluate the pharmacokinetics and bioequivalence of a newly developed generic formulation of S-1 in Chinese cancer patients in comparison with the branded reference formulation of S-1.

Methods: A single-dose, randomized-sequence, open-label, two-way self-crossover study was conducted in 30 Chinese cancer patients. The subjects alternatively received the two formulations (40 mg/m^2, *po*) with a 7-d interval. Plasma concentrations of FT, CDHP, Oxo, and 5-Fu were determined using LC-MS/MS. Pharmacokinetic parameters, including C_{max}, T_{max}, $t_{1/2}$, AUC_{0-t}, and $AUC_{0-\infty}$ were determined using non-compartmental models with DAS2.0 software. Bioequivalence of the two formulations were to be evaluated according to 90% CIs for the log-transformed ratios of AUC and C_{max} of S-1. Adverse events were evaluated through monitoring the symptom, physical and laboratory examinations, ECGs and subject interviews.

Results: The mean values of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ of FT, 5-Fu, CDHP, and Oxo for the two formulations had no significant differences. The 90% CIs for natural log-transformed ratios of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were within the predetermined bioequivalence acceptance limits. A total of 11 mild adverse events, including fatigue, nausea and vomiting, anorexia, diarrhea and myelosuppression, were observed, and no serious and special adverse events were found.

Conclusion: The newly developed generic formulation and reference formulation of S-1 have similar pharmacokinetics with one dose (40 mg/m^2) in Chinese cancer patients. Both the formulations of S-1 are well tolerated.

Keywords: anticancer drug; S-1; tegafur; 5-fluorouracil; 5-chloro-2,4-dihydroxypyridine; potassium oxonate; pharmacokinetics

Acta Pharmacologica Sinica (2013) 34: 570–580; doi: 10.1038/aps.2012.169; published online 11 Feb 2013

Introduction

5-Fluorouracil (5-Fu) has been widely prescribed for solid tumors since it was first introduced by Heidelberger *et al* in 1957[1]. 5-Fu, through its incorporation, exerts cytotoxic effects on tumor cells through thymidylate synthase inhibition and modification of RNA. However, because 5-Fu is catabolized by the activity of dihydropyrimidine dehydrogenase (DPD), up to 90% of the administered 5-Fu is metabolized to fluoroalanine, hindering the drug's antitumor effect[2]. To overcome this drawback, S-1 has been developed as a novel oral fluorouracil antitumor drug and has been termed a "self-rescuing drug". In this oral formulation, fluoropyrimidine is combined with three pharmacological agents: tegafur (FT), which is a prodrug of 5-Fu; 5-chloro-2,4-dihydroxypyridine (CDHP), which inhibits DPD activity; and potassium oxonate (Oxo), which is a poorly absorbed inhibitor of orotate phosphoribosyl transferase. S-1 is administered as a capsule in which FT, CDHP and Oxo are combined at a molar ratio of 1:0.4:1, with each capsule containing 20 or 25 mg of FT. When FT is combined with CDHP, which is 180-fold more potent than uracil for inhibiting DPD *in vitro*, biologically relevant plasma 5-Fu concentrations are sustained in both plasma and in tumors[3–5]. S-1 was rapidly absorbed from the gastrointestinal tract, with tegafur and 5-Fu plasma concentrations peaking at 1.5 and 3 h post-treatment, respectively[6]. Hirata *et al* reported that after oral administration of S-1, the plasma concentration of 5-Fu was similar to that obtained with a continuous intravenous infusion of 5-Fu[7]. The rationale for Oxo as a constituent of S-1 is its potential to reduce gastrointestinal toxicity by inhibiting orotate phosphoribosyl transferase and subsequent 5-Fu phosphorylation or activation in gastrointestinal tissues[5, 8]. Therefore, Oxo may reduce gastrointestinal toxicity without interfering with the antitumor activity of 5-Fu.

# These authors contributed equally to this article.
* To whom correspondence should be addressed.
E-mail zhoncology@suda.edu.cn
Received 2012-10-08 Accepted 2012-11-07

Initially, S-1 was developed as an oral anticancer drug for the treatment of gastric cancer in Japan. Now, accumulating evidence has demonstrated that S-1 has a potent antitumor effect not only in gastric cancer but also in a broad range of malignancies, including squamous cell carcinoma of the head and neck, colorectal cancer, cholangiocarcinoma, breast cancer, non-small-cell lung cancer and pancreatic cancer[9–16]. The antitumor activity of S-1 has been established in various experimental models including rodent tumor models and human xenograft models[3, 5, 9, 17]. In studies where the antitumor effects of S-1 and Uracil-Tegafur UFT were compared, S-1 demonstrated superior activity against human gastric, colorectal, and breast cancer xenografts[7, 18–20]. S-1 demonstrated less toxicity than 5-Fu when administered as a protracted infusion[21]. The cytotoxic action of S-1 is ultimately exerted by 5-Fu through its antimetabolic effects on DNA (through thymidylate synthase inhibition) and RNA levels. Its toxicity profile is manageable and similar to that of other fluoropyrimidines with regard to gastrointestinal adverse events (diarrhea, nausea, vomiting) and myelosuppression (neutrocytopenia, thrombocytopenia, anemia). However, Hand-Foot Syndrome, which is often observed with continuous infusion of 5-Fu and capecitabine, is not a major problematic toxicity associated with S-1[22].

Although the pharmacokinetics of S-1 and oral 5-Fu formulations were previously described in other countries, the pharmacokinetic profile of S-1 in a Chinese population has not been described[7, 23, 24]. The State Food and Drug Administration (SFDA) requires a bioequivalence study for the marketing of a newly developed generic formulation in China. Therefore, the present study was designed to assess the pharmacokinetics of a test (Minsheng Pharmaceutical Co, Ltd, Hangzhou, China) and branded reference (Taiho Pharmaceutical Co, Ltd, Japan) formulation of S-1 capsules in Chinese cancer patients.

Materials and methods

Study design and procedures

This was a single-dose, randomized-sequence, open-label, two-way, self-crossover study conducted at the Department of Oncology, the Second Affiliated Hospital of Soochow University (Suzhou, China) from April 2010 to September 2010. The study (Chinese National Registry Code: 2005L01593) was performed in accordance with the latest version of the World Medical Association Declaration of Helsinki[25], International Conference on Harmonisation Guideline for Good Clinical Practice[26], and the local regulatory guidelines of the SFDA of China[27]. The study protocol and informed consent form were approved by the independent ethics and research committee at the Second Affiliated Hospital of Soochow University prior to initiation of the study. Before undergoing any study procedures, all participants provided written informed consent after they had been informed of the study's purpose, nature, procedures, and risks by the clinical investigators.

Eligible subjects were randomly assigned using a computer-generated random number table (1:1 ratio) to odd and even numbers. The odd subjects received the test formulation (Minsheng Pharmaceutical Co, Ltd, Hangzhou, China) and were then administered the reference formulation (Taiho Pharmaceutical Co, Ltd, Japan) after an interval of 7 d. In contrast, the even subjects received the reference formulation and then the test formulation after an interval of 7 d. Subjects were orally administered the test or the reference formulations with 150-200 mL of warm water half an hour after breakfast on the test day. During the test, the subjects were prohibited from smoking, taking medications, and consuming food or beverages containing alcohol, caffeine, or tea. The subjects were under close medical monitoring. Adverse reactions were carefully observed in the confinement of the hospital unit for 48 h after drug administration, and the patients were discharged after the last blood sample was drawn and tolerability assessments were performed. Appropriate rescue equipment and medicines were prepared in case of serious adverse events. After a washout period of 7 d, the subjects returned to the unit, and the alternate formulation was administered following the same protocol.

Subjects

All of the subjects enrolled in this study met the following conditions: (1) they were Chinese cancer patients with gastrointestinal cancer, non-small-cell lung cancer, head and neck cancer, unresectable or recurrent breast cancer, or pancreatic cancer diagnosed cytologically or histologically; (2) they had a Karnofsky Performance Scale (KPS) score ≥70 and an expected life span ≥3 months; (3) the age range was 35–65 years; (4) the white blood cell (WBC) count was $\geq 3.8\times10^9$/L, the absolute neutrophil count (ANC) was $\geq 1.5\times10^9$/L, the platelet (PLT) count was $\geq 100.0\times10^9$/L, and the hemoglobin (Hb) level was ≥90 g/L; (5) serum bilirubin was not more than the upper limit of the normal value, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (AKP) were not more than 2.5 times the upper limit of the normal value, and creatinine was not more than 1.25 times the upper limit of the normal value; (6) no clinically significant abnormalities were present before the test, as determined by 12-lead ECGs, heart function analysis, and urinalysis; (7) the patients were not undergoing any other antitumor therapy; (8) the patients had not undergone chemotherapy for 2 weeks prior to the study and had not taken any trial drugs for 4 weeks prior to the test; and (9) the subjects had not taken any drugs possibly related to the test for 2 weeks prior to the test, such as 5-fluorouracil derivatives containing 5-Fu, UFT, tegafur, doxifluridine, capecitabine, carmofur, folinate, and the pyrimidine antifungal agents, fluorocytosine, sorivudine and brivudine.

The subjects were not admitted into this study if they met any of the following conditions: (1) they were pregnant or breast-feeding women or there were no effective contraceptive measures for subjects in their reproductive period; (2) they displayed mental disorders, brain metastases or meningeal metastasis; (3) the subjects had serious or uncontrolled internal diseases or infections; (4) the subjects suffered from one or more failures of a major organ, including heart, lung, liver, renal failure; (5) the subjects took other trial drugs or partici-

pated in other clinical tests simultaneously; (6) the subjects displayed symptomatic peripheral neuropathy and the NCI (National Cancer Institute) score was ≥II grade; (7) the subjects had a known allergy to 5-Fu; or (8) the subject was unable to take oral medicine.

The subjects were removed from this study if any of the following occurred: (1) they displayed poor compliance or were unable to take the medicine on schedule; (2) they could not sustain participation in the clinical trial and requested removal; (3) no any related record was available; (4) blood samples were not collected at the proper time; (5) the processing, preservation, or transportation of blood samples was defective; or (6) drugs which could potentially affect the pharmacokinetic results were simultaneously used.

Materials and reagents

Reference standards for tegafur (FT) (lot No 100300220; purity, 99.86%), 5-chloro-2,4-dihydroxypyridine (CDHP) (lot No 070627; purity, 99.96%), potassium oxonate (Oxo) (lot No 070828; purity, 99.97%), and 5-fluorouracil (5-Fu) (lot No 0902249; purity, 101.4%) were donated by Minsheng Pharmaceutical Co, Ltd, China (Hangzhou, China). The internal standards (IS) for nicotinamide and [$^{13}C_2$,$^{15}N_3$] potassium oxonate were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) and Toronto Research Chemicals Inc (Toronto, Canada), respectively. Methanol, acetic acid, ammonium acetate, and methanoic acid were HPLC grade and purchased from Sigma-Aldrich China, Shanghai Sigma-Aldrich Trading, Ltd (Shanghai, China), Tedia Company Inc (Fairfield, Ohio, USA), and Fluka Company Inc (Seelze, Germany), respectively. 4-Bromomethyl-7-methoxycoumarin and 18-crown-6-ether were HPLC grade and purchased from Tokyo Kasei Kogyo Co, Ltd (Tokyo, Japan). Potassium carbonate, N,N-dimethylformamide, acetoacetate, ammonia, water, and hydrochloric acid were of analytical grade and purchased from Sinopharm Chemical Reagent Co Ltd (Shanghai, China).

Drug formulations

The test formulation (lot No C09L901; expiration date Nov 2011) was manufactured by Minsheng Pharmaceutical Co, Ltd, China (Hangzhou, China). The reference formulation (lot No 9G98B; expiration date Jun 2012) was manufactured by Taiho Pharmaceutical Co, Ltd, Japan (Tokyo, Japan). Both formulations, donated by Minsheng Pharmaceutical Co, Ltd, were from commercially available batches with valid certificates of analysis and were kept in a sealed container at a controlled room temperature of 15 °C to 25 °C until further use. Both formulations contained 20 mg of drug per pill (FT content) and contained 20 mg FT, 5.8 mg CDHP and 19.6 mg Oxo. The administration dose of both of formulations was 40 mg per square meter of body surface per time according to the results of the corresponding phase II clinical trial.

Blood sampling

Blood samples (5 mL) were collected from a suitable forearm vena mediana by an immediate venipuncture or by an indwelling catheter at the following time points: 0 (before administration), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, and 48 h after administration. Prior to each sample collection, 1 mL of blood was drawn and discarded. Blood samples were drawn into pretreated heparin-containing tubes, and plasma samples were separated within 30 min after drawing by centrifugation at 1000×*g* for 10 min at 4 °C. Plasma was stored frozen (-80 °C) in labeled tubes until analysis by a liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) method at the Shanghai Institute of Materia Medica, Chinese Academy of Sciences (Shanghai, China). After a 7-d washout period, the subjects received the alternate formulation, and blood samples were again drawn and analyzed using the same protocol.

Quantification of plasma concentrations of FT, 5-Fu, and CDHP by an LC-MS/MS method

Frozen human plasma samples were thawed at ambient temperature. An LC-MS/MS method was used to simultaneously measure the level of FT, 5-Fu, and CDHP in plasma after the sample proteins had been precipitated by methanol. A 50 μL aliquot of plasma, 50 μL of the internal standard working solution (2 μg/mL nicotinamide) and 250 μL of methanol were mixed in a centrifuge tube. The mixture was vortexed for 1 min and then centrifuged at 16000×*g* for 5 min at 4 °C. The supernatant layer was transferred to a clean dry centrifuge tube, and the sample was evaporated to dryness under a stream of nitrogen at 40 °C. The dry residue was reconstituted with 100 μL of mobile phase (methanol:water:ammonia water:acetic acid, 27:73:0.18×10^{-2}:0.18×10^{-1}), and a 20 μL aliquot of the sample was injected onto the analytical column for LC-MS/MS analysis.

During the pre-study validation, calibration curves for the analytes in human plasma were obtained using 7 calibration standards (12, 30, 75, 240, 600, 1500, and 3000 ng/mL for FT and 2, 5, 12.5, 40, 100, 250, and 500 ng/mL for 5-Fu and CDHP), each of which were freshly prepared in-house, in duplicate, and extracted together with blank plasma samples and quality control (QC) samples for each analytic run. QC samples of 30, 300, and 2700 ng/mL of FT and 5, 50, and 450 ng/mL of 5-Fu and CDHP, which were prepared in-house on the day the first study samples and were received and stored frozen (-20 °C) together with the study samples, were used to assess the intra- and interday precision (RSD), accuracy (RE), recovery, and stability. No peaks interfering with quantitation were observed throughout the validation process. For FT, the intra- and interday precisions were <7.7% and <13.1% for the 3 calibration standards, the extraction recovery was 97.2% to 107%, and the average accuracy was -4.7% to 3.3%. For 5-Fu, the intra- and interday precisions were <7.1% and <13.3% for the 3 calibration standards, the extraction recovery was 83.4% to 93.5%, and the average accuracy was 1.7% to 8.6%. For CDHP, the intra- and interday precisions were <8.4% and <12.1% for the 3 calibration standards, the extraction recovery was 100% to 106%, and the average accuracy was -4.4% to 1.2%.

An LC system (Shimadzu Scientific Corp, Kyoto, Japan) equipped with 2 pumps (model LC20ADvp), an autosampler (SIL-HTA), and a controller module was used to perform the chromatographic separation on a C18 guard column (4 mm×3.0 mm, Phenomenex, Los Angeles, CA, USA) and a Synergi 4u Hydro-RP 80A chromatographic column (150 mm×4.6 mm, 4 μm, Phenomenex, Los Angeles, CA, USA). A tandem mass spectrometer (API 4000, Applied Biosystems, Foster City, CA, USA) equipped with electrospray ionization (ESI) and atmospheric pressure chemical ionization was operated in positive-ionization mode. Analyst version 1.4.1 software (Applied Biosystems, Foster City, CA, USA) was used for instrument control and data processing.

Multiple reaction monitoring analysis was applied to detect ion transitions at *m/z* 198→41, *m/z* 127→40, *m/z* 144→100, and *m/z* 122→78 for FT, 5-Fu, CDHP, and the internal standard nicotinamide, respectively. The retention times for FT, 5-Fu, CDHP, and nicotinamide were 6.71, 3.70, 7.11, and 4.23 min, respectively. The peak area was measured for calculation of the peak area ratio of the analytes to their corresponding IS, and the plasma concentrations were estimated. Using these conditions, the method of measuring the levels of FT, 5-Fu, and CDHP in subjects' plasma by LC-MS/MS was established.

Quantification of plasma concentrations of Oxo by an LC-MS/MS method

A 100-μL aliquot of plasma, 20 μL of an internal standard working solution (500 ng/mL [$^{13}C_2$,$^{15}N_3$]-Oxo), and 50 μL of methanol were mixed. The precipitate was obtained by the addition of 300 μL of methanol, and the supernatant layer was transferred to a 10 mL centrifuge tube. Plasma samples were incubated in a reaction with hydrochloric acid at 60 °C for 20 min. The products were blown dry with nitrogen at 40 °C at the end of incubation, and the derivatization agents (4-bromomethyl-7-methoxycoumarin and 18-crown-6-ether dissolved in 10 mL N,N-dimethylformamide) and kalium carbonicum were added to the residue to incubate for 1 h at 60 °C. After extraction with ethyl acetate, the concentration of Oxo was determined by an LC-MS/MS method similar to that used for quantification of the plasma concentrations of FT, 5-Fu, and CDHP.

The chromatographic column was a Zorbax SB-C18 column (150 mm×4.6 mm, 5 μm, Agilent, Palo Alto, CA, USA), and the precolumn was a C18 guard column (4 mm×3 mm, Phenomenex, Los Angeles, CA, USA). The mobile phase was methanol: 20 mmol/L ammonium acetate: formic acid (70:30:0.3). The ion source was an atmospheric pressure chemical ionization (APCI) source, and the scanner mode was multiple reaction monitoring. Ion transitions occurred at *m/z* 492.1→259.2 for the Oxo derivative and at *m/z* 494.6→262.4 for the [$^{13}C_2$,$^{15}N_3$]-Oxo derivative. The retention times of the Oxo and [$^{13}C_2$,$^{15}N_3$]-Oxo derivatives were 6.51 and 6.47 min, respectively. The linear concentration range of Oxo was 2.00 to 150 ng/mL. For the Oxo derivative, the intra-and interday precisions were <10.0% and <8.5% for the 3 calibration standards (5.00, 30.0, 120 ng/mL), the extraction recovery was 67.4% to 76.5%, and the average accuracy was -4.1% to -0.9%.

These results indicated that the established LC-MS/MS method was valid and suitable for this study. The samples from each individual subject were analyzed in independent experiments.

Tolerability assessments

The subjects were observed by clinicians, nurses, and clinical pharmacists during the entire study. Tolerability was evaluated by monitoring vital signs (including body temperature, breathing rate, blood pressure and heart rate), physical examinations, clinical laboratory examination (including hematology, urinalysis, liver and renal function), and 12-lead ECGs at the beginning and end of each study period. Blood pressure and heart rate were measured in the sitting position using a calibrated mercurial sphygmomanometer, and body temperature was taken with a mercury thermometer in the armpit. The 12-lead ECGs were recorded and reported by an unbiased and skilled ECG specialist. All laboratory examinations were performed blind to the subjects at the Clinical Laboratory of the Second Affiliated Hospital of Soochow University, which is suitable for providing daily clinical laboratory examinations for patients.

The subjects were also questioned regarding the occurrence of adverse events (AEs) associated with drug administration, such as fatigue, nausea, vomiting, anorexia, diarrhea, *etc*. It was considered a serious AE (SAEs) if the subject died, a life-threatening emergency, or endured a prolonged hospital stay leading to disability or requiring medical intervention to prevent permanent impairment or damage. All SAEs and AEs were recorded in the original data record and on the case-report form. The relationship between SAEs or AEs and the two formulations was determined by investigators who were blind to the study schedule.

Pharmacokinetic and statistical analysis

Pharmacokinetic parameters were analyzed by non-compartmental models using the DAS2.0 software (Drug and Statistics, Wannan Medical College, Wuhu, China). Plasma drug concentration–time curves were drawn, and the C_{max} and T_{max} were derived from these curves. AUC_{0-t} (area under the plasma concentration-time curve) was calculated according to the linear trapezoidal rule[28]. $AUC_{0-\infty}$ was calculated as follows: $AUC_{0-\infty}=AUC_{0-t}+C_t/k_e$, where C_t was the last measured concentration at time t, and k_e was the slope of the linear regression of the log-transformed concentration–time curve. Plasma $t_{1/2}$ was calculated as $0.693/k_e$[27]. Descriptive statistics, including the means (SD), were used to summarize the pharmacokinetic data for the two formulations. Relative bioavailability was calculated as follows: $F=AUC_{0-t\ (test)}/AUC_{0-t\ (reference)}\times 100\%$.

Statistical analysis was performed using the SPSS (Statistical Package for the Social Sciences) version 13.0 software package for Macintosh (SPSS, Inc, Chicago, IL, USA). ANOVA was used to analyze the natural logarithm (ln)-transformed pharmacokinetic parameters (AUC_{0-t}, $AUC_{0-\infty}$, and C_{max}) by general

linear model procedures. The bioequivalence between the test and reference formulations was evaluated based on the C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$. The nonparametric signed rank test was used to compare the T_{max} values for the two formulations. Two-way ANOVA for a 2×2 crossover design was used to assess the effects of the formulations, the period, the sequence, and the subjects based on log-transformed C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ data[29]. The ratios of the log-transformed C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ values of the two formulations were calculated, and the 90% confidence intervals (CIs) were obtained. The probability of exceeding the limits of acceptance was determined by two one-sided *t*-tests[29]. According to the guidelines of the SFDA of China[27], the two formulations were considered to be bioequivalent if the 90% CIs of the test/reference ratios of AUC were within the predetermined bioequivalence range of 80% to 125% and the C_{max} was between 70% and 143%. A value of $P<0.05$ was considered statistically significant, and $P<0.01$ was remarkably significant.

Results

Demographic data

A total of 30 Chinese cancer patients that met the inclusion criteria (18 male and 12 female; mean [SD] age, 54 [7] years [range, 38–64 years]; weight, 62.4 [7.6] kg [range, 43–75 kg]; height, 164.5 [6.9] cm [range, 153–175 cm]; and body surface area, 1.69 [0.12] m^2 [range, 1.46–1.90 m^2]) were enrolled and completed this study. These subjects included 11 cases of gastric cancer, 9 cases of rectal cancer, 3 cases of colon cancer, 3 cases of unresectable or recurrent breast cancer, 1 case of non-small-cell lung cancer, 1 case of laryngeal pharynx cancer, 1 case of esophageal carcinoma and 1 case of tongue cancer, which were diagnosed cytologically or histologically. All the subjects were included in the pharmacokinetic, bioequivalence and tolerability assessments.

Tolerability

No clinically significant abnormalities on physical examination, including vital sign measurements or ECG recordings, were observed. The AEs of the two formulations mainly manifested in the common side effects of the fluorouracil chemotherapeutic drugs, including fatigue, nausea and vomiting, anorexia, diarrhea, and myelosuppression. The AEs of the two formulations are summarized in Table 1. All AEs were transient, considered by the investigators to be mild and did not need clinical intervention or were treated symptomatically. The incidence of AEs for the test formulation was not statistically different from the reference formulation. This was considered by the investigators to be related to the side effects caused by the fluorouracil chemotherapeutic drugs themselves and not to the test or reference formulation. Other possible adverse effects, such as interstitial pneumonitis, dental ulcer, rash, headache, bleeding, liver function damage, acute renal failure, anosphrasia, dyspnea, were not reported. No serious or unpredictable AEs were reported, and none of the subjects were withdrawn from the study due to AEs. This study showed that the two formulations performed good security with one dose (40 mg/m^2).

Pharmacokinetic properties

The mean plasma concentration-time curves of FT, 5-Fu, CDHP, and Oxo after administration of a single oral dose of 40 mg/m^2 of the two formulations to 30 Chinese cancer patients are shown in Figure 1A–1D. The main pharmacokinetic parameters (AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, and $t_{1/2}$) for both formulations are listed in Table 2. The analyses of variance of the pharmacokinetic parameters (AUC_{0-t}, $AUC_{0-\infty}$, C_{max}) are summarized in Table 3. There were no significant differences between the two formulations regarding AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, or $t_{1/2}$ by two-paired *t*-test and in T_{max} by Wilcoxon signed rank test ($P>0.05$, shown in Table 4). No period or sequence effects were detected for any pharmacokinetic properties by ANOVA; however, a significant individual effect was observed for AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} ($P<0.05$). The mean relative bioavailabilities (F) of FT, 5-Fu, CDHP, and Oxo in the test and reference formulation were 98.3%±23.3%, 95.5%±22.7%, 97.5%±21.1%, and 124.0%±99.3%, respectively.

Bioequivalence evaluation

The 90% CIs of the test/reference ratios of the natural log-transformed values of AUC_{0-t}, and $AUC_{0-\infty}$ (as an indication of the extent of absorption) of FT, 5-Fu, CDHP, and Oxo were within the predetermined bioequivalence range of 80% to 125%, and C_{max} (as an indication of the rate of absorption) was within the range of 70% to 143% (Table 5). Therefore, it

Table 1. Adverse events after administration of the test or reference formulation of oral antitumor drug S-1 in Chinese patients with cancer (n=30).

Adverse events	Subject No (%) (Test*)	Subject No (%) (Reference@)	Severity&	Action	*P* value
Fatigue	1 (3.3%)	1 (3.3%)	Mild	No intervention	>0.05
Anorexia	1 (3.3%)	1 (3.3%)	Mild	No intervention	>0.05
Nausea and vomiting	1 (3.3%)	3 (10%)	Grade I–II	Antiemetic drug	>0.05
Diarrhea	1 (3.3%)	1 (3.3%)	Grade I	Antidiarrheic drug	>0.05
Myelosupp-ression	0	1 (3.3%)	Grade I	No intervention	>0.05

* Test S-1: Manufactured by Minsheng Pharmaceutical Co, Ltd, Hangzhou, China

@ Reference S-1: TS-1 (Taiho Pharmaceutical Co, Ltd, Tokushima Plant, Japan).

& The severity was graded according to WHO grading standards for common toxicity of anticancer drugs of 2005.

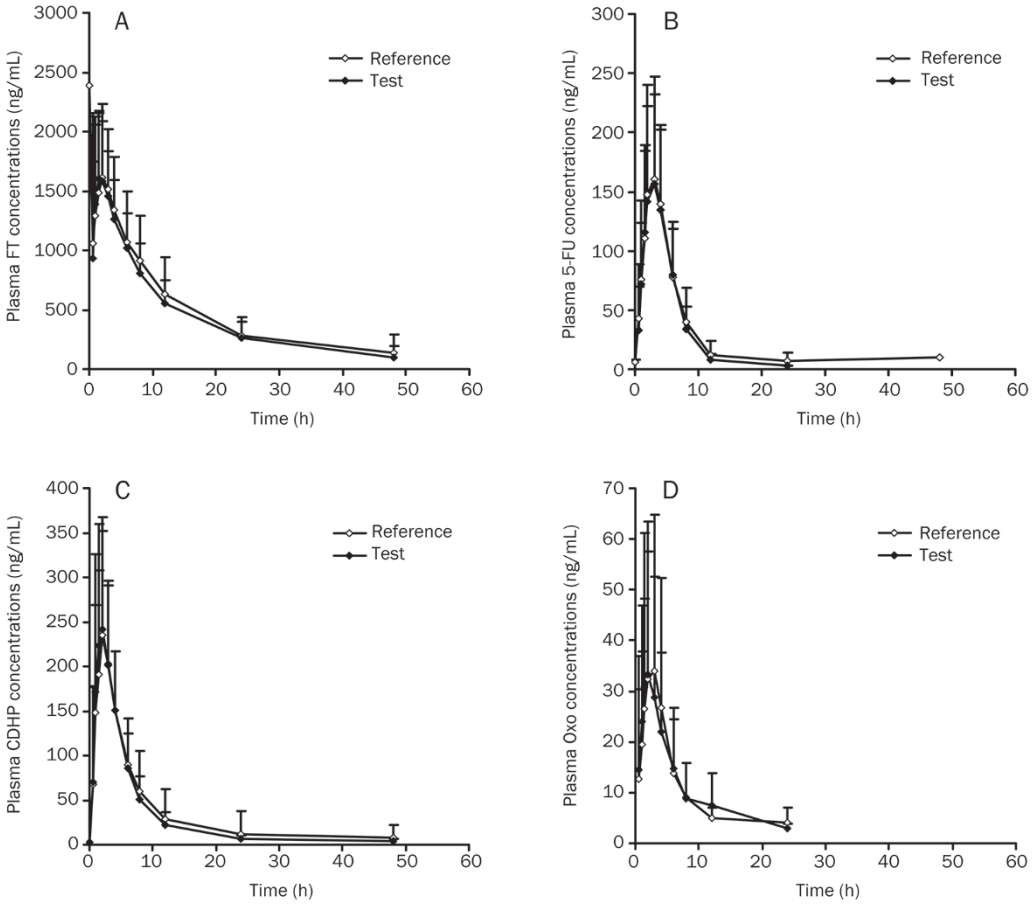

Figure 1. Mean (SD) plasma concentration-time curves of (A) FT, (B) 5-Fu, (C) CDHP and (D) Oxo after administration of a single oral dose of 40 mg/m^2 of test (manufactured by Minsheng Pharmaceutical Co, Ltd, Hangzhou, China) and reference (Taiho Pharmaceutical Co, Ltd, Tokushima Plant, Japan) formulations in Chinese cancer patients (n=30).

was deduced that the test and reference formulations were bioequivalent according to the guidelines of the SFDA of China.

Discussion

According to Chinese SFDA guidelines (2005), measurements of every effective component for compound preparation are generally recommended for bioequivalence studies. S-1 is a fixed combination of CDHP, Oxo, and tegafur that is converted *in vivo* to 5-Fu. Therefore, the bioequivalence studies for S-1 should detect the pharmacokinetic parameters of FT, CDHP, Oxo, and 5-Fu. In many studies, gas chromatography-mass spectrometry has been used to determine 5-Fu, CDHP, and Oxo, and high performance liquid chromatography (HPLC) or HPLC-UV is often used for FT detection[7, 23, 24, 30]. Our study and others have adopted liquid chromatography-tandem mass spectrometry (LC-MS/MS) to measure all analytes[31]. Although the cost of LC-MS/MS is higher than that of other determination methods, it provides more reliable and accurate information regarding relative molecular mass and structure, simplifies the test procedure, and saves sample preparation and analysis time as a result of the reliability of LC for segregation analysis and the sensitivity of MS for identification and structural analysis.

Tegafur is a prodrug of the cytotoxic agent 5-Fu and is mainly converted in the liver through hydroxylation by cytochrome P-450 2A6 (CYP2A6)[32]. Ikeda and Kajita *et al* showed that the formation of 5-Fu from tegafur was inhibited more than 90% and 82% in human liver microsomes using a CYP2A6-selective antibody and complementary DNAs expressing human CYPs, respectively[32, 33]. The C_{max} and AUC_{0-t} were 1869.7 (479.4) ng/mL and 21.0 (7.1) μg/mL·h, respectively, after administration of a single oral dose of 40 mg/m^2 of S-1 in Chinese cancer patients in the present study; this is higher than that for Caucasian subjects and is similar to that for Japanese subjects treated with similar doses of S-1[7, 23]. Conversion of tegafur to 5-Fu proceeds faster in Westerners because the C_{max} and AUC_{0-10} for 5-Fu were approximately 40%–50% higher than the corresponding values in Japanese subjects treated with similar doses of S-1[19]. Although these differences in the pharmacokinetic parameter profiles remain unexplained, it is postulated that the different efficacies of the CYP2A6 enzymes in Westerners and Easterners may contribute to the pharmacokinetic differences[34]. Polymorphisms of

Table 2. Pharmacokinetic parameters of FT, 5-Fu, CDHP, and Oxo after administration of an oral dose of single 40 mg/m^2 of test and reference formulations in Chinese patients with cancer (n=30).

Ingredient	Parameter	Test*	Reference@
FT	AUC_{0-t} (μg/mL·h)	21.0 (7.1)	22.7 (10.1)
	$AUC_{0-\infty}$ (μg/mL·h)	22.4 (8.4)	24.2 (10.8)
	$T_{1/2}$ (h)	10.9 (3.1)	10.8 (3.5)
	T_{max} (h)	1.9 (1.2)	3.6 (8.5)
	C_{max} (ng/mL)	1869.7 (479.4)	1901.0 (778.4)
5-Fu	AUC_{0-t} (ng/mL·h)	838.7 (359.7)	916.6 (412.6)
	$AUC_{0-\infty}$ (ng/mL·h)	863.4 (357.8)	967.6 (437.8)
	$T_{1/2}$ (h)	2.0 (0.8)	3.2 (5.3)
	T_{max} (h)	3.0 (1.0)	3.1 (1.3)
	C_{max} (ng/mL)	170.9 (77.6)	175.1 (83.7)
CDHP	AUC_{0-t} (ng/mL·h)	1382.5 (625.5)	1489.3 (756.7)
	$AUC_{0-\infty}$ (ng/mL·h)	1402.1 (628.2)	1626.9 (1259.1)
	$T_{1/2}$ (h)	3.9 (1.9)	4.8 (3.8)
	$_{max}$ (h)	2.0 (0.9)	2.2 (1.0)
	C_{max} (ng/mL)	286.6 (130.7)	261.8 (112.9)
Oxo	AUC_{0-t} (ng/mL·h)	189.7 (149.1)	184.8 (143.5)
	$AUC_{0-\infty}$ (ng/mL·h)	213.1 (154.8)	207.9 (142.2)
	$T_{1/2}$ (h)	3.5 (1.8)	3.5 (3.7)
	T_{max} (h)	2.4 (1.2)	2.3 (1.1)
	C_{max} (ng/mL)	38.8 (30.0)	40.7(31.4)

* Test S-1: Manufactured by Minsheng Pharmaceutical Co, Ltd, Hangzhou, China.
@ Reference S-1: TS-1 (Taiho Pharmaceutical Co, Ltd, Tokushima Plant, Japan).

Table 3. Analysis of variance of the pharmacokinetic parameters of FT, 5-Fu, CDHP, and Oxo after administration of an oral dose of single 40 mg/m^2 of test and reference formulations in Chinese patients with cancer (n=30).

Ingredient	Parameter	*F* value		
		Inter-formulation	Inter-period	Inter-individual
FT	AUC_{0-t}	0.992	0.836	6.872
	$AUC_{0-\infty}$	1.170	1.509	7.068
	C_{max}	0.115	0.124	7.179
5-Fu	AUC_{0-t}	2.431	1.131	9.606
	$AUC_{0-\infty}$	2.319	0.247	5.918
	C_{max}	0.065	8.780	17.228
CDHP	AUC_{0-t}	1.246	1.918	7.332
	$AUC_{0-\infty}$	1.465	2.190	5.073
	C_{max}	2.814	0.005	10.103
Oxo	AUC_{0-t}	0.005	0.034	4.600
	$AUC_{0-\infty}$	0.002	0.037	6.529
	C_{max}	0.056	0.014	2.383

Notes: F0.05 (1, 28)=4.20, F0.05 (29, 28)=1.88.

CYP2A6 are thought to result in differences in CYP2A6 activity that lead to the distinct pharmacokinetics of S-1.

Table 4. Wilcoxon signed rank test of T_{max} of FT, 5-Fu, CDHP, and Oxo after administration of an oral dose of single 40 mg/m^2 of test and reference formulations in Chinese patients with cancer (n=30).

Ingredient	Parameter	Test*	Reference@	*P* value
FT	Mean (SD)	1.90 (1.23)	3.63 (8.50)	>0.05
	Max-Min	6.00–0.50	48.00–0.50	
	Median	1.5	2	
5-Fu	Mean (SD)	3.00 (0.98)	3.08 (1.32)	>0.05
	Max-Min	6.00–2.00	6.00–0.50	
	Median	3	3	
CDHP	Mean (SD)	2.03 (0.90)	2.17 (1.08)	>0.05
	Max-Min	4.00–1.00	6.00–0.50	
	Median	2	2	
Oxo	Mean (SD)	2.40 (1.18)	2.35 (1.15)	>0.05
	Max-Min	6.00–0.50	6.00–0.50	
	Median	2	2	

* Test S-1: Manufactured by Minsheng Pharmaceutical Co, Ltd, Hangzhou, China.
@ Reference S-1: TS-1 (Taiho Pharmaceutical Co, Ltd, Tokushima Plant, Japan).

Table 5. Comparison of 90% CIs of natural log(ln)-transformed parameters of FT, 5-Fu, CDHP, and Oxo for an oral dose of single 40 mg/m^2 of test and reference formulations in Chinese patients with cancer (n=30).

Ingredient	Parameter	Test*	Reference@	90% CI
FT	AUC_{0-t}	3.546	5.537	87.6%–103.5%
	$AUC_{0-\infty}$	3.338	5.502	86.9%–103.2%
	C_{max}	7.791	7.049	94.9%–108.2%
5-Fu	AUC_{0-t}	2.831	5.949	84.7%–100.7%
	$AUC_{0-\infty}$	2.089	5.135	81.9%–101.1%
	C_{max}	6.290	6.742	91.8%–106.6%
CDHP	AUC_{0-t}	3.062	5.295	86.0%–103.2%
	$AUC_{0-\infty}$	2.133	4.554	82.3%–103.3%
	C_{max}	7.301	3.897	99.9%–118.9%
Oxo	AUC_{0-t}	1.965	1.822	82.5%–123.2%
	$AUC_{0-\infty}$	2.308	2.397	84.7%–117.0%
	C_{max}	1.856	2.312	76.6%–122.3%

* Test S-1: Manufactured by Minsheng Pharmaceutical Co, Ltd, Hangzhou, China.
@ Reference S-1: TS-1 (Taiho Pharmaceutical Co, Ltd, Tokushima Plant, Japan).

5-Fu is a long-standing and commonly used antitumor agent, and its antitumor activity is due to inhibitory effects on thymidylate synthase and DNA synthesis as well as antimetabolic effects on RNA. However, it is difficult to maintain effective concentrations of 5-Fu in the plasma and tumorous tissue because 5-Fu is rapidly degraded by dihydropyrimidine dehydrogenase[35, 36]. Many methods of 5-Fu administration have been explored in an effort to improve efficacy. S-1 contains the 5-Fu prodrug tegafur and two enzyme inhibitors, CDHP and potassium oxonate. Because CDHP inhibits

dihydropyrimidine dehydrogenase activity and Oxo suppresses pyrimidine phosphoribosyl transferase activity, oral S-1 administration can generate higher and more prolonged serum concentrations of 5-Fu than protracted intravenous injection of 5-Fu without increasing the incidence of adverse events in the gastrointestinal tract[3, 7]. In the pharmacokinetic analysis of 5-Fu levels in human tumor cells using ^{19}F-labeled nuclear magnetic resonance, it has been reported that the pharmacokinetics in blood correlates with that in target tumors[37]. Therefore, measuring the plasma 5-Fu level is important for determining its effect and safety. The elimination rate of 5-Fu from the patient body is dependent on the rate of conversion of FT to 5-Fu and the extent of inhibition of CDHP on dihydropyrimidine dehydrogenase. In our study, after administration of a single oral dose of 40 mg/m^2 of S-1 in Chinese cancer patients, the C_{max}, AUC_{0-t}, $t_{1/2}$, and t_{max} of 5-Fu were 170.9 (77.6) ng/mL, 838.7 (359.7) μg/mL·h, 2.0 (0.8) and 3.0 (1.0) h, respectively, which suggests that the 5-Fu plasma concentration after S-1 oral administration is almost equal to or higher than the 5-Fu concentration after continuous venous infusion. Therefore, oral S-1 is potentially an attractive alternative for delivering protracted venous 5-Fu.

CDHP is an inhibitor of DPD, the rate-limiting enzyme of 5-Fu metabolism. When combined with tegafur, CDHP can potentially maintain prolonged 5-Fu concentrations in the plasma and in tumors by competitively inhibiting DPD in a reversible manner[3]. It has been reported that the half-life of 5-Fu is 2 to 4 h for S-1 and 40 min for a previous generation UFT in which uracil was used to inhibit DPD; therefore, CDHP has 180-fold higher DPD inhibitory activity than that of uracil *in vitro*[7]. Ahmed *et al* found that the inhibitory activity of uracil was still efficacious for several weeks after drug withdrawal, but the efficacy of CDPH was reversible and the metabolism of 5-Fu was accelerated *in vivo* after drug discontinuation[38]. Therefore, the risk of serious side effects induced by 5-Fu accumulation if S-1 is discontinued and substituted with other fluorouracil anticancer drug is minimal. CDHP is known to predominantly undergo urinary excretion through glomerular filtration, and approximately 50% of CDHP is found in the urine[7, 39]. Thus, a patient's renal function should be monitored in clinical trials to avoid the incidence of serious adverse events such as myelosuppression, as an increased plasma concentration of 5-Fu results from the accumulation of CDHP in patients with renal dysfunction[40]. In our study, the renal function of all the enrolled patients was monitored throughout, and no cases of renal dysfunction were observed.

Oxo is a specific orotate phosphoribosyltransferase (ORTC) inhibitor and has the potential to reduce 5-Fu-related gastrointestinal toxicity through its inhibition of ORTC and 5-Fu phosphorylation[5, 8]. Animal experiments revealed that the output of fluorouridine monophosphate (FUMP) and 5-Fu incorporated into RNA decreased by approximately 70% only in the small intestine, whereas the output decreased by 0%–20% in the bone marrow and tumor regions[8]. This suggests that Oxo is distributed at high levels in the digestive tract after S-1 oral administration, leading to relief of gastrointestinal toxicity induced by 5-Fu. Therefore, oral S-1 administration can generate higher and more prolonged serum concentrations of 5-Fu than protracted intravenous injection of 5-Fu without increasing the incidence of adverse events concerning the gastrointestinal tract[3, 7]. With regard to Oxo, it is worth mentioning that if its concentration is excessively high in the plasma, Oxo could be taken up by tumor tissues and intercept 5-Fu phosphorylation, resulting in a reduced 5-Fu-related antitumor effect. In our patient population, the C_{max} and AUC_{0-t} of Oxo were 38.8 (30.0) ng/mL and 189.7 (149.1) ng/mL·h, respectively, while the plasma concentration of Oxo was 0.8 nmol/g after S-1 oral administration (110 mg/Kg) in the animal experiments[8]. Mende *et al* investigated the influence of food on the pharmacokinetics of S-1, and their results showed that the C_{max} and AUC_{0-t} of Oxo were significantly increased on an empty stomach, which suggests that Oxo was distributed at high levels in the gastrointestinal tract on an empty stomach[41]. Thus, it should be clinically recommended that S-1 be orally given on an empty stomach to increase Oxo bioavailability and reduce 5-Fu-related gastrointestinal toxicity. Our study was designed for S-1 to be taken orally 30 min after eating food to avoid the contribution of food intake on the plasma concentration of Oxo.

The purpose of this study was to determine whether an S-1 test formulation was equivalent to its reference. Two drug formulations are generally considered to be equivalent if they have identical rates and extents of absorption in the same molar dose and under similar experimental conditions. In the present study, the analysis of variance (ANOVA) showed that there were no significant differences in 30 subjects between the two formulations on the pharmacokinetic parameters tested (AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, $t_{1/2}$) for FT, 5-Fu, CDHP, and Oxo (P>0.05). However, a significant subject individual effect was obtained for AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} (P<0.05). The 90% CIs of the test/reference ratios of AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} for FT, 5-Fu, CDHP, or Oxo were located within the bioequivalence criteria range (80%–125% for AUC and 70%–143% for C_{max})[27]. Therefore, this study documented that the test and reference formulations of S-1 are bioequivalent according to the guidelines of the SFDA of China. In general, equivalent formulations are considered to be therapeutically equivalent[42, 43].

S-1 was remarkably well tolerated and was designed to provide a continuous plasma 5-Fu exposure comparable to prolonged iv infusion with less gastrointestinal toxicity and fewer incidences of Hand–Foot Syndrome. In clinical studies, the majority of patients had only mild gastrointestinal symptoms and/or hematologic toxicity. In addition to neutropenia, the incidence rate of grade III or greater or other side effects was less than 10%[44]. There was a correlation between the AUC of 5-Fu and the response or toxicity. The nontoxic concentration of 5-Fu was determined to be 1.5 mmol/L, 195 ng/mL, or less because the steady-state concentration (Css) of 5-Fu correlated with incidence of leucopenia[45–47]. Hematological toxicity was the main toxicity for Japanese patients, but diarrhea was more prominent for Western patients. Although these differences in

the toxicity profiles remain unexplained, it is postulated that different efficacies of the CYP2A6 enzymes in Westerners and Japanese may contribute to pharmacokinetic variability, which cause differences in toxicity[31]. When S-1 was combined with cisplatin, gemcitabine or other chemotherapeutic drugs, the toxicity was remarkably increased[48, 49]. In this study, the AEs of the two formulations mainly manifested as mild fatigue, myelosuppression, nausea and vomiting, anorexia and diarrhea. No serious AEs or unpredictable effects were reported, which suggested that the two formulations were safe at the tested dose. However, the subjects had only taken the test or reference formulation once in the present study. Therefore, the side effects of S-1 should be closely monitored in clinical use, especially for additive toxicity when combined with other drugs.

5-Fu is a long-standing and widely prescribed antitumor agent for a variety of tumor indications, and S-1 has similar prospects as a 5-Fu derivative. S-1 is widely used as a standard option for chemotherapy in patients with gastric cancer in Japan[39]. Thus far, S-1 has been approved worldwide for head and neck cancer, colorectal cancer, non-small-cell lung, breast cancer, pancreatic cancer and cholangiocarcinoma[9–16]. Although S-1 has been confirmed to have antitumor effects according to the existing clinical data, the optimal combined chemotherapy with S-1 and whether S-1 can improve long-term overall survival are unknown. Clinical trials are being conducted to determine the effects of combined chemotherapy containing S-1, for example a phase II study of a combined therapy of gemcitabine and oral S-1 for cholangiocarcinoma[50]. Other clinical trials are underway to explore the antitumor effects of S-1 in cervical cancer, thymic carcinoma, and tongue cancer, among others[51–53]. At present, the conventional S-1 monotherapy involves oral administration of S-1 at 40 mg/m^2 twice a day for 28 d followed by a 2-week rest period. The relatively long half-life of the active compound, 5-Fu, may allow for once daily dosing, and this possibility should be evaluated in future studies[24].

Conclusions

This small study found that the test and reference formulations have similar pharmacokinetics based on one dose (40 mg/m^2) in Chinese cancer patients. Both formulations were generally well tolerated in the population studied.

Acknowledgements

The authors would like to thank the volunteers and staff who participated in this study. The authors would like to thank the sponsor, Minsheng Pharmaceutical Co, Ltd, China (Hangzhou, China), for providing financial support. Mr Qin WAN and Xiu-gui CHEN who are employees of Minsheng Pharmaceutical Co, Ltd of China provided significant assistance during the clinical trials. This work was supported by grants from Suzhou City Research Foundation for Applied Basic Research (No YJS0930 and No SYS201129), Natural Science Foundation of the Jiangsu Higher Education Institutions of China (No 10KJB320020), National Natural Science Foundation of China (No 81000944) and Research Foundation for "Reserved Academic Leader" from the Second Affiliated Hospital of Soochow University.

Author contribution

Zhi-xiang ZHUANG designed and managed the study; Hong ZHU, Ji WANG, Min-gao ZHU, Hui WANG, Wang-yang PU, Hua-hui BIAN, Lei CHEN, and Hong ZHANG performed the study; Ji WANG, Min-gao ZHU, and Hui WANG analyzed the data; Hong ZHU wrote and revised the manuscript.

References

1 Heidelberger C, Chaudhuri NK, Danneberg P, Mooren D, Griesbach l, Duschinsky R, *et al*. Fluorinated pyrimidines, a new class of tumour-inhibitory compounds. Nature 1957; 179: 663–6.
2 Heggie GD, Sommadossi JP, Cross DS, Huster WJ, Diasio RB. Clinical pharmacokinetics of 5-fluorouracil and its metabolites in plasma, urine, and bile. Cancer Res 1987; 47: 2203–6.
3 Shirasaka T, Shimamato Y, Ohshimo H, Yamaguchi M, Kato T, Yonekura K, *et al*. Development of a novel form of an oral 5-fluorouracil derivative (S-1) directed to the potentiation of the tumor selective cytotoxicity of 5-fluorouracil by two biochemical modulators. Anticancer Drugs 1996; 7: 548–57.
4 Tatsumi K, Fukushima M, Shirasaka T, Fujii S. Inhibitory effects of pyrimidine, barbituric acid and pyridine derivatives on 5-fluorouracil degradation in rat liver extracts. Jpn J Cancer Res 1987; 78: 748–55.
5 Takechi T, Nakano K, Uchida J, Mita A, Toko K, Takeda S, *et al*. Antitumor activity and low intestinal toxicity of S-1, a new formulation of oral tegafur, in experimental tumor models in rats. Cancer Chemother Pharmacol 1997; 39: 205–11.
6 Chu QS, Hammond LA, Schwartz G, Ochoa L, Rha SY, Denis L, *et al*. Phase I and pharmacokinetic study of the oral fluoropyrimidine S-1 on a once-daily-for-28-day schedule in patients with advanced malignancies. Clin Cancer Res 2004; 10: 4913–21.
7 Hirata K, Horikoshi N, Aiba K, Okazaki M, Denno R, Sasaki K, *et al*. Pharmacokinetic study of S-1, a novel oral fluorouracil antitumor drug. Clin Cancer Res 1999; 5: 2000–5.
8 Shirasaka T, Shimamoto Y, Fukushima M. Inhibition by oxonic acid of gastrointestinal toxicity of 5-fluorouracil without loss of its antitumor activity in rats. Cancer Res 1993; 53: 4004–9.
9 Fujii M, Tomita K, Nishijima W, Tsukuda M, Hasegawa Y, Ishitoya J, *et al*. Phase I/II study of S-1 plus cisplatin combination chemotherapy in patients with advanced/recurrent head and neck cancer. Jpn J Clin Oncol 2010; 40: 214–21.
10 Nakayama T, Morita S, Takashima T, Kamigaki S, Yoshidome K, Ito T, *et al*. Phase I study of S-1 in combination with Trastuzumab for HER2-positive metastatic breast cancer. Anticancer Res 2011; 31: 3035–9.
11 Sano M, Saeki T, Takashima S, Horikoshi N, Miura S, Morimoto K, *et al*. Late phase II study of S-1 in patients with advanced and/or recurrent breast cancer (Meeting Abstracts). J Clin Oncol 2000; 18: 404.
12 Koizumi W, Boku N, Yamaguchi K, Miyata Y, Sawaki A, Kato T, *et al*. Phase II study of S-1 plus leucovorin in patients with metastatic colorectal cancer. Ann Oncol 2010; 21: 766–71.
13 Sasaki T, Isayama H, Nakai Y, Mizuno S, Yamamoto K, Yagioka H, *et al*. Multicenter phase II study of S-1 monotherapy as second-line chemotherapy for advanced biliary tract cancer refractory to gemcitabine. Invest New Drugs 2012; 30: 708–13.
14 Koizumi W, Kurihara M, Nakano S, Hasegawa K. Phase II study of S-1, a novel oral derivative of 5-fluorouracil, in advanced gastric cancer.

For the S-1 Cooperative Gastric Cancer Study Group. Oncology 2000; 58: 191–7.
15 Niitani H, Kawahara M, Segawa Y. Activity of S-1 in patients with non-small-cell lung cancer. Proc Am Soc Clin Oncol 2000; 19: 1995.
16 Okusaka T, Funakoshi A, Furuse J, Boku N, Yamao K, Ohkawa S, *et al*. A late phase II study of S-1 for metastatic pancreatic cancer. Cancer Chemother Pharmacol 2008; 61: 615–21.
17 Konno H, Tanaka T, Baba M, Kanai T, Matsumoto K, Kamiya K, *et al*. Therapeutic effect of 1 M tegafur-0.4 M 5-chloro-2,4-dihydroxypyridine-1 M potassium oxonate (S-1) on liver metastasis of xenotransplanted human colon carcinoma. Jpn J Cancer Res 1999; 90: 448–53.
18 Fukushima M, Satake H, Uchida J, Shimamoto Y, Kato T, Takechi T, *et al*. Preclinical antitumor efficacy of S-1: a new oral formulation of 5-fluorouracil on human tumor xenografts. Int J Oncol 1998; 13: 693–8.
19 Van Groeningen CJ, Peters GJ, Schornagel JH, Gall H, Noordhuis P, de Vries MJ, *et al*. Phase I clinical and pharmacokinetic study of oral S-1 in patients with advanced solid tumors. J Clin Oncol 2000; 18: 2772–9.
20 Hoff PM, Wenske C, Medgyesy D, Royce ME, Brito R, Zukowski TH, *et al*. Phase I and pharmacokinetic study of the novel oral fluoropyrimidine, S-1. Proc Am Soc Clin Oncol 1999; 18: 173.
21 Fukushima M, Shimamoto Y, Kato T, Uchida J, Yonekura R, Ohshimo H, *et al*. Anticancer activity and toxicity of S-1, an oral combination of tegafur and two biochemical modulators, compared with continuous iv infusion of 5-fluorouracil. Anticancer Drugs 1998; 9: 817–23.
22 Saif MW, Syrigos KN, Katirtzoglou NA. S-1: a promising new oral fluoropyrimidine derivative. Expert Opin Investig Drugs 2009; 18: 335–48.
23 Peters GJ, Noordhuis P, Van Kuilenburg AB, Schornagel JH, Gall H, Turner SL, *et al*. Pharmacokinetics of S-1, an oral formulation of ftorafur, oxonic acid and 5-chloro-2,4-dihydroxypyridine (molar ratio 1:0.4:1) in patients with solid tumors. Cancer Chemother Pharmacol 2003; 52: 1–12.
24 Hoff PM, Saad ED, Ajani JA, Lassere Y, Wenske C, Medgyesy D, *et al*. Phase I study with pharmacokinetics of S-1 on an oral daily schedule for 28 days in patients with solid tumors. Clin Cancer Res 2003; 9: 134–42.
25 World Medical Association Declaration of Helsinki (WMA). Ethical principles for medical research involving human subjects. Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000. http://www.wma.net/en/30publications/10policies/b3/index.html. Accessed January 25, 2008.
26 European Agency for the Evaluation of Medical Products (EMEA), International Conference on Harmonisation-World Health Organization. Guideline for Good Clinical Practice. ICH topic E6. Geneva, Switzerland: WHO; 2002. http://www.emea.europa.eu/pdfs/human/ich/013595en.pdf.
27 State Food and Drug Administration (SFDA), center for drug evaluation. Guideline for bioavailability and bioequivalence studies of generic drug products. http://www.cde.org.cn/zdyz.do?method=largePage&id=2066.
28 Chow SC, Liu JP. Design and analysis of bioavailability and bioequivalence studies. Third Edition. CRC Press. Boca Raton, FL, USA. 2009. p 1–30.
29 Schuirmann DJ. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. J Pharmacokinet Biopharm 1987; 15: 657–80.
30 Cohen SJ, Leichman CG, Yeslow G, Beard M, Proefrock A, Roedig B, *et al*. Phase I and pharmacokinetic study of once daily oral administration of S-1 in patients with advanced cancer. Clin Cancer Res 2002; 8: 2116–22.
31 Ajani JA, Faust J, Ikeda K, Yao JC, Anbe H, Carr KL, *et al*. Phase I pharmacokinetic study of S-1 plus cisplatin in patients with advanced gastric carcinoma. J Clin Oncol 2005; 23: 6957–65.
32 Ikeda K, Yoshisue K, Matsushima E, Nagayama S, Kobayashi K, Tyson CA, *et al*. Bioactivation of tegafur to 5-fluorouracil is catalyzed by cytochrome P-450 2A6 in human liver microsomes *in vitro*. Clin Cancer Res 2000; 6: 4409–15.
33 Kajita J, Fuse E, Kuwabara T, Kobayashi H. The contribution of cytochrome P450 to the metabolism of tegafur in human liver. Drug Metab Pharmacokinet 2003; 18: 303–9.
34 Shimada T, Yamazaki H, Guengerich FP. Ethnic-related differences in coumarin 7-hydroxylation activities catalyzed by cytochrome P450 2A6 in liver microsomes of Japanese and Caucasian populations. Xenobiotica 1996; 26: 395–403.
35 Malet-Martino M, Martino R. Clinical studies of three oral prodrugs of 5-fluorouracil (capecitabine, UFT, S-1): a review. Oncologist 2002; 7: 288–323.
36 Diasio RB, Lu Z. Dihydropyrimidine dehydrogenase activity and fluorouracil chemotherapy. J Clin Oncol 1994; 12: 2239–42.
37 Wolf W, Presant CA, Servis KL, el-Tahtawy A, Albright MJ, Barker PB, *et al*. Tumor trapping of 5-fluorouracil: *in vivo* ^{19}F NMR spectroscopic pharmacokinetics in tumor-bearing humans and rabbits. Proc Natl Acad Sci U S A 1990; 87: 492–6.
38 Ahmed FY, Johnston SJ, Cassidy J, O'Kelly T, Binnie N, Murray GI, *et al*. Eniluracil treatment completely inactivates dihydropyrimidine dehydrogenase in colorectal tumors. J Clin Oncol 1999; 17: 2439–45.
39 Fujita K, Nakayama H, Ichikawa W, Yamamoto W, Endo H, Nagashima F, *et al*. Pharmacokinetics of 5-fluorouracil in elderly Japanese patients with cancer treated with S-1 (a combination of tegafur and dihydropyrimidine dehydrogenase inhibitor 5-chloro-2,4-dihydroxypyridine). Drug Metab Dispos 2009; 37: 1375–7.
40 Ikeda M, Furukawa H, Imamura H, Shimizu J, Ishida H, Masutani S, *et al*. Pharmacokinetic study of S-1, a novel oral fluorouracil antitumor agent in animal model and in patients with impaired renal function. Cancer Chemother Pharmacol 2002; 50: 25–32.
41 Mende B, Krauss J, Thyssen D, Kredtke S, Scheulen ME, Strumberg D, *et al*. Pharmacokinetic study of S-1. Int J Clin Pharmacol Ther 2009; 47: 65–7.
42 Chow S, Liu P. Design and analysis of bioavailability and bioequivalence studies. Marcel Dekker, New York. 1992.
43 Committee for proprietary medicinal products (CPMP). Note for guidance on the investigation of bioavailability and bioequivalence. London. 1998.
44 Shirasaka T. Development history and concept of an oral anticancer agent S-1 (TS-1): its clinical usefulness and future vistas. Jpn J Clin Oncol 2009; 39: 2–15.
45 Hillcoat BL, Mcculloch PB, Figueredo AT, Ehsan MH, Rosenfeld JM. Clinical response and plasma levels of 5-fluorouracil in patients with colonic cancer treated by drug infusion. Br J Cancer 1978; 38: 719–24.
46 Van Groeningen CJ, Pinedo HM, Heddes J, Kok RM, De Jong AP, Wattel E, *et al*. Pharmacokinetics of 5-fluorouracil assessed with a sensitive mass spectrometric method in patients on a dose escalation schedule. Cancer Res 1988; 48: 6956–61.
47 Au JL, Rustum YM, Ledesma EJ, Mittelman A, Creaven PJ. Clinical pharmacological studies of concurrent infusion of 5-fluorouracil and thymidine in treatment of colorectal carcinomas. Cancer Res 1982;

42: 2930–7.
48 Koizumi W, Narahara H, Hara T, Takagane A, Akiya T, Takagi M, *et al*. S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial. Lancet Oncol 2008; 9: 215–21.
49 Nakamura K, Yamaguchi T, Sudo K, Hara T, Denda T, Azemoto R, *et al*. A phase II trial of oral S-1 combined with gemcitabine in patients with unresectable biliary tract cancer. ASCO Meeting Abstracts 2009; 27: e15527.
50 Takashima A, Morizane C, Ishii H, Nakamura K, Fukuda H, Okusaka T, *et al*. Randomized phase II study of gemcitabine plus S-1 combination therapy vs. S-1 in advanced biliary tract cancer: Japan Clinical Oncology Group Study (JCOG0805). Jpn J Clin Oncol 2010; 40: 1189–91.
51 Katsumata N, Hirai Y, Kamiura S, Sugiyama T, Kokawa K, Hatae M, *et al*. Phase II study of S-1, an oral fluoropyrimidine, in patients with advanced or recurrent cervical cancer. Ann Oncol 2011; 22: 1353–7.
52 Koizumi T, Agatsuma T, Komatsu Y, Kubo K. Successful S-1 monotherapy for chemorefractory thymic carcinoma. Anticancer Res 2011; 31: 299–301.
53 Nakayama M, Hayakawa K, Okamoto M, Niibe Y, Ishiyama H, Kotani S. Phase I/II trial of concurrent use of S-1 and radiation therapy for T2 glottic cancer. Jpn J Clin Oncol 2010; 40: 921–6.