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Circadian variations in the pharmacokinetics of capecitabine and its metabolites in rats

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ABSTRACT

Capecitabine, an orally available prodrug of 5-fluorouracil, is widely used to treat patients with colorectal cancer. Although various studies have shown circadian variations in plasma 5-fluorouracil concentrations during long-term infusion, it is still unknown whether circadian variations also exist following administration of capecitabine. The present study aimed to investigate whether the pharmacokinetics of capecitabine and its metabolites, including 5-fluorouracil, vary according to administration time in rats. Rats were orally administered capecitabine (180 mg/kg) at 07:00 (23 h after light onset, HALO), 13:00 (5 HALO), or 19:00 h (11 HALO). Plasma concentrations of capecitabine and its metabolites, such as 5′-deoxy-5-fluorocytidine (5′-DFCR), 5′-deoxy-5-fluorouridine (5′-DFUR), and 5-fluorouracil, were determined after capecitabine administration. The results showed that the $t_{1/2}$ and $AUC_{0-\infty}$ values of 5-fluorouracil differed as a function of the dosing time of capecitabine. The maximum and minimum mean $t_{1/2}$ values of 5-fluorouracil were obtained when the drug was administered at 07:00 h (23 HALO: 3.1 ± 1.2 h) and 13:00 h (5 HALO: 1.5 ± 0.6 h), respectively. The $AUC_{0-\infty}$ value of 5-fluorouracil at 07:00 h (23 HALO: 533.9 ± 195.7 μmol·h/L) was 1.8-fold higher than the value at 13:00 h (5 HALO: 302.5 ± 157.1 μmol·h/L). The clearance of 5-fluorouracil followed a cosine circadian curve, and the simulated population mean clearance was highest at rest times and lowest during active times in rats. The results for the plasma 5′-DFCR and 5′-DFUR levels indicated that circadian variations in the sequential metabolism of capecitabine to 5-fluorouracil would also affect plasma 5-fluorouracil levels following capecitabine administration. In conclusion, the pharmacokinetics of capecitabine and its metabolites, including 5-fluorouracil, varied according to time of dosing, suggesting that the capecitabine administration time is an important factor in achieving sufficient efficacy and reducing toxicity in patients.

1. Introduction

The anticancer agent 5-fluorouracil is the cornerstone in regimens for the treatment of metastatic colorectal cancer (Saif et al., 2009; Lee et al., 2016). Modern regimens such as the folinic acid, 5-fluorouracil, and oxaliplatin (FOLFOX) combination and the folinic acid, 5-fluorouracil, and irinotecan (FOLFIRI) combination consist of long-term 5-fluorouracil infusion. High inter- and intra-individual variations in the pharmacokinetics of 5-fluorouracil have yet to be investigated, despite being a major contributor to treatment failure (Saif et al., 2009). Because circadian rhythm is considered a potential factor affecting these pharmacokinetic variations, chronomodulated regimens based on the 5-fluorouracil dosing according to circadian rhythm has been attempted in order to reduce toxicity and improve therapeutic outcomes (Milano and Chamorey, 2002; Saif et al., 2009). Lévi et al. (1994) conducted a randomized clinical trial comparing the fixed-rate infusion of oxaliplatin, 5-fluorouracil, and leucovorin chemotherapy to chronomodulated administration of the same chemotherapy regimen in patients with metastatic colorectal cancer. The findings showed that chronomodulated chemotherapy appeared to reduce toxicity and improve efficacy compared to the fixed-rate infusion (Lévi et al., 1994, 1997). However, one issue that remains unresolved is how best to determine the peak time for optimal dosing strategy. Several studies have investigated circadian variation in plasma 5-fluorouracil levels during continuous infusion, and most did not find consistent times of day for peak and trough (Fleming et al., 2015). With regard to a clinical application of the chronomodulated chemotherapy, the identification of circadian patterns associated with the pharmacokinetics of 5-fluorouracil in individual patients receiving infusion remains a challenge (Saif et al., 2009).

Capecitabine, an orally available prodrug of 5-fluorouracil, is widely used to treat patients with colorectal cancer (Petrelli et al., 2012; Watanabe et al., 2017). Similar to the FOLFOX and FOLFIRI regimen, the capecitabine plus oxaliplatin (XELOX) regimen is accepted

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as a standard treatment for advanced or metastatic colorectal cancer. After oral administration, capecitabine is absorbed into the blood and sequentially converted to 5-fluorouracil via a three-step enzyme activation process in the liver and tissues (Desmoulin et al., 2002; Shindoh et al., 2006; Shindoh et al., 2011): 1) Capecitabine is converted to 5′-deoxy-5-fluorocytidine (5′-DFCR) by carboxylesterase, mainly in the liver. 2) 5′-DFCR is converted to 5′-deoxy-5-fluorouridine (5′-DFUR) by cytidine deaminase, primarily in the liver. 3) 5′-DFUR is converted to 5-fluorouracil by pyrimidine nucleoside phosphorylase, which is highly expressed in many organs and has greater activity in tumor tissues than normal tissues. This unique conversion enables the replacement of long-term infusion of 5-fluorouracil in chemotherapy. It has been reported that capecitabine-based chemotherapy is significantly more cost-effective than chemotherapy involving long-term 5-fluorouracil infusion (Lee et al., 2016).

Similar to chronomodulated chemotherapy involving long-term infusion of 5-fluorouracil, the chronomodulated XELOX regimen has been proposed as a way to reduce toxicity, which often requires dose reduction and subsequently, loss of antitumor effects (Santini et al., 2007; Qvortrup et al., 2010; Akgun et al., 2014; Pilancı et al., 2016). However, conflicting results have been reported. Qvortrup et al. (2010) found that the chronomodulated XELOX regimen did not reduce toxicity or improve efficacy in patients with advanced colorectal cancer in a randomized study. In contrast, Pilancı et al. (2016) recently conducted a phase II study to assess the benefits of capecitabine morning and noon dosing as part of a first-line XELOX regimen in patients with metastatic colorectal cancer. Findings of good tumor control and favorable toxicity profiles led the authors to conclude that the chronomodulated regimen could be a therapeutic option in the first-line treatment of metastatic colorectal cancer. In order to determine the utility of the chronomodulated XELOX regimen, the evaluation of circadian variations in the pharmacokinetics (i.e., chronopharmacokinetics) of capecitabine remains a critical challenge (Qvortrup et al., 2010; Pilancı et al., 2016). Nevertheless, to our knowledge, no studies have investigated the circadian variations associated with the pharmacokinetics of capecitabine and its metabolites. In contrast to direct continuous infusion of 5-fluorouracil, it is possible that the conversion of capecitabine to 5-fluorouracil, via three enzymatic steps, generates no circadian variations in plasma 5-fluorouracil levels due to counteracting effects among the 5-fluorouracil-producing and -eliminating enzymes.

In the current study, we evaluated whether the pharmacokinetics of capecitabine and its metabolites varied according to drug dosing time in rats.

2. Materials and methods

2.1. Chemicals

Capecitabine was purchased from LKT Laboratories, Inc. (St. Paul, MN, USA). 5′-DFCR and 5′-DFUR were obtained from Toronto Research Chemicals (Toronto, ON, Canada). 5-Fluorouracil was supplied by Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and 5-bromouracil was purchased from Sigma-Aldrich Co. (Steinheim, Germany). All other reagents were of analytical grade and were used without further purification.

2.2. Animals

Male Wistar rats (10 weeks of age) were supplied by Nippon SLC Co., Ltd. (Hamamatsu, Japan). All rats were housed in a 12-h light/dark cycle (lights on from 08:00 h to 20:00 h) in a temperature-controlled room for at least 5 days. Free access to food and water were permitted, and the rats were fasted over 12 h prior to the experiments. All experimental procedures using animals were approved by an institutional review board and performed in accordance with the Kyoto Pharmaceutical University Guidelines for Animal Experimentation.

2.3. Pharmacokinetics study of capecitabine and its metabolites in rats

A total of 18 rats were divided into 3 groups ($n = 6$ in each group). Capecitabine was dissolved in 1% carboxymethyl-cellulose sodium in distilled water at each circadian time assessed in the study. A single dose of 180 mg/kg capecitabine (22.5 mg/mL) was orally administered to rats at different times of the day, 07:00 (23 h after light onset [HALO]), 13:00 (5 HALO), or 19:00 h (11 HALO). The dose of capecitabine was selected based on results from a previous study using rats (Shindoh et al., 2006). The dosing time was also determined based on our previous study investigating circadian variations associated with the pharmacokinetics of 5-fluorouracil after intravenous administration (Kobuchi et al., 2013, 2016) and theses times correspond to the rest-activity cycle. Blood samples (250 μL) were collected from the external left jugular vein at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, and 8 h following drug administration. All blood samples were transferred to heparinized centrifuge tubes and centrifuged immediately at 14,000 × *g* for 15 min. The obtained plasma samples were stored at − 80 °C until used for analysis.

2.4. Assay

Plasma concentrations of capecitabine, 5′-DFCR, 5′-DFUR, and 5-fluorouracil were determined using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The assay was performed according to a previously reported method (Montange et al., 2010; Deenen et al., 2013) with minor modifications. Saturated ammonium acetate (150 μL) was added to the standard and unknown plasma samples (100 μL) containing 10 μL of I.S. (5-bromouracil, 25 μg/mL in 50% methanol), and mixed vigorously for 15 s. Acetic acid/isopropyl alcohol (1 mL; 10:1, *v*/v) was added, vortexed for over 30 s, and centrifuged for 15 min at 14,000 × *g*. After centrifugation, the supernatant was transferred to a fresh centrifuge tube and evaporated to dryness under a stream of nitrogen at 60 °C. The mobile phase (100 μL) was added to the resulting residue and vortexed for over 30 s. The reconstituted solution (30 μL) was injected into the LC–MS/MS system for determining the concentration of capecitabine and 5-FU. To evaluate 5′-DFCR and 5′-DFUR levels, the obtained reconstituted solution (10 μL) was diluted with mobile phase (90 μL). This diluted solution was injected (30 μL) into the LC–MS/MS system, which comprised an API 3200 triple-quadrupole mass spectrometer equipped with a turbo ion spray sample inlet as an interface for electrospray ionization (ESI), an LC-10AD micropump (Shimadzu Corp., Kyoto, Japan), and an AS8020 automatic sample injector (Toso, Tokyo, Japan). The mobile phase was acetonitrile/0.1% formic acid (20:80, v/v) for capecitabine and 5-FU, and acetonitrile/10 mM ammonium acetate (50:50, v/v) for 5′-DFCR and 5'DFUR, respectively. The flow rate was set at 0.2 mL/min and the column used was COSMOSIL® HILIC Packed column (2.0 × 150 mm, 5 μm; Nacalai Tesque, Inc., Kyoto, Japan) maintained at 50 °C. The ion spray voltage was 5500 V for capecitabine and 5-FU, and − 4500 V for 5′-DFCR and 5'DFUR. The source temperature was set at 550 °C. SRM analyses were conducted with transition *m/z* 360.0 → 130.0 for capecitabine; 131.0 → 114.0 for 5-FU; and 191.0 → 174.0 for the I.S. in the positive ion mode, and *m/z* 244.0 → 128.0 for 5′-DFCR; 245.0 → 129.0 for 5′-DFUR; and 188.9 → 42.0 for the I.S. in the negative ion mode. The lower limit of quantification (LLOQ) for the all analytes was < 0.01 μg/mL in 100 μL of plasma sample. Each calibration curve was linear over LLOQ with a correlation coefficient (r^2) of > 0.99.

2.5. Pharmacokinetic analysis

To obtain pharmacokinetic parameters for capecitabine and its metabolites, non-compartmental pharmacokinetic analysis was performed using the non-compartmental analysis program of the WinNonlin® software (version 6.3, Pharsight Co., Mountain View, CA, USA). The time (T_{max}) at which plasma concentrations reached a