Association between circadian and chemotherapeutic cycle effects on plasma concentration of 5-fluorouracil and the clinical outcome following definitive 5-fluorouracil/cisplatin-based chemoradiotherapy in patients with esophageal squamous cell carcinoma

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Abstract. Therapeutic drug monitoring (TDM) of 5-fluorouracil (5-FU) is believed to be a clinical option for improving clinical responses. Evaluating the potential factors contributing to plasma 5-FU concentration is important to develop TDM of 5-FU. Our aim was to evaluate the association of the circadian and treatment cycle effects on plasma 5-FU concentration with the clinical response. A post hoc population analysis was performed using the plasma concentration of 5-FU and clinical response data, including prognosis from 49 patients with esophageal squamous cell carcinoma after treatment with definitive 5-FU/cisplatin-based chemoradiotherapy, consisting of prolonged infusion of 5-FU at 400 mg/(m²·day) for 5 days. The circadian rhythm and treatment cycle were applied as covariates to the model equation. The plasma 5-FU concentration in the evening was 1.3-fold higher compared with the morning, and in the second cycle, it was 1.5-fold increased compared with the first cycle, with relatively small inter-individual variations (23.3 and 16.8%). Clinical efficacy depended on the plasma 5-FU concentration, excluding the covariate effects (P=0.025), which correlated with age and height but not body surface area. Circadian variation did not contribute to the clinical response, and the increase in 5-FU plasma concentration in the second cycle significantly correlated with leucocyte counts obtained before chemoradiotherapy. The higher plasma concentration of 5-FU in the early phase of treatment may be the key determinant of clinical efficacy, whereas the variations in the plasma concentration of 5-FU owing to the time of day and treatment cycle are small contributors.

Introduction

The anticancer agent 5-fluorouracil (5-FU) is well known as the key drug in regimens for the treatment of gastrointestinal cancer (1,2). Large inter- and intraindividual variations in the pharmacokinetics of 5-FU have been investigated, and this high pharmacokinetic variation is one of the factors contributing to treatment failure (1). Monitoring 5-FU concentration in plasma (known as therapeutic drug monitoring (TDM)) is proposed to improve clinical outcomes, including alleviation of 5-FU toxicity (2). However, identification of the factors contributing to the plasma 5-FU concentration, target plasma levels, and appropriate blood sampling time in individual chemotherapeutic regimens remains a challenge.

In a previous study by our research group, Japanese patients with esophageal squamous cell carcinoma (ESCC) were followed for 5 years after treatment with definitive 5-FU/cisplatin (CDDP)-based chemoradiotherapy and the relation between prognosis and the plasma 5-FU concentration was evaluated (3,4). The chemoradiotherapy consisted of the continuous infusion of 5-FU at 400 mg/(m²·day) for 5 days in weeks 1 and 2, and plasma concentrations of 5-FU were determined at eight sampling points up to the end of a second course. We found that the plasma concentration of 5-FU is a possible key determinant of the clinical response in patients with ESCC after the treatment with definitive 5-FU/CDDP-based chemoradiotherapy. However, circadian variations in the plasma 5-FU concentration were observed during continuous infusion of 5-FU, and the repeated treatment cycle also affected the plasma 5-FU concentration, thereby complicating the analysis of the degree to which these factors affect plasma 5-FU concentrations. These effects make it difficult to analyze the data (5

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and to determine appropriate blood sampling time points and plasma 5-FU levels. In our previous study, to normalize the circadian rhythm and the treatment cycle effects on plasma 5-FU concentration, an eight-point average value was used to investigate the association with the clinical response (3). To realize a personalized 5-FU/CDDP-based chemoradiotherapy regimen based on the plasma 5-FU concentration to improve the clinical response, more detailed analysis of the circadian rhythm and the treatment cycle effects on the plasma 5-FU concentration has remained an important issue for further research.

Recently, we successfully evaluated the circadian variations of 5-FU after intravenous administration of 5-FU or oral administration of capecitabine, which is a prodrug of 5-FU, in rats using population model analysis (6,7). One of the advantages of population analysis is the quantitative evaluation of covariate effects on the drug plasma concentrations. The results of these studies could point to a new chronomodulated schedule for the administration of capecitabine (7). Therefore, population analysis is considered effective at evaluating the factors contributing to plasma concentrations of 5-FU.

In the current study, we performed population analysis to evaluate the effects of the circadian rhythm and treatment cycle on the plasma 5-FU concentration in patients with ESCC after treatment with definitive 5-FU/CDDP-based chemoradiotherapy.

Materials and methods

The data source. The 5-FU plasma concentration and clinical outcome data from 49 patients with ESCC after treatment with definitive 5-FU/CDDP-based chemoradiotherapy in our previous study (3) were used as the source of data for population analysis. A summary of the patients' characteristics is shown in Table I. The treatment protocol consisted of the prolonged infusion of 5-FU at 400 mg/(m^2·day) for days 1-5 and 8-12, the infusion of CDDP at 40 mg/(m^2·day) on days 1 and 8, and radiation at 2 Gy/day on days 1 to 5, 8 to 12, and 15 to 19, with a second course repeated after a 2-week interval. Blood samples were collected at eight sampling points (on day 3 at 17:00 h, day 4 at 05:00 h, day 10 at 17:00 h, and day 11 at 05:00 h in the first cycle, and day 38 at 17:00 h, day 39 at 05:00 h, day 45 at 17:00 h, and day 46 at 05:00 h in the second course). In the current study, the plasma sample on day 1 and day 2 could not be obtained from patients because the sample collection time was limited during definitive 5-FU/CDDP-based chemoradiotherapy. The sampling in the early h of the infusion could underestimate the plasma concentration-time curve (AUC) value of 5-FU due to a wide variety of factors, including fluctuating rates of 5-FU metabolism before steady-state conditions are reached and collecting before the infusion pump was fully primed with drug (8). Moreover, it is necessary to minimize the effects of high plasma CDDP levels on plasma concentrations of 5-FU. Based on the half-life of CDDP (as unbound plasma concentration of platinum) in distribution and elimination phase is approximately 31.2 min and 20.1 h (9,10), respectively, day 3 and 4 in the regimen was considered to be appropriate sampling time to analyze the circadian and treatment cycles effects of plasma concentration of 5-FU. Further details of these data including

Table I. Demographic and clinicopathologic characteristics and clinical outcome.

Characteristics	Values
Demographic and clinicopathologic characteristics	
Male/female	46/3
Age, year[a]	64.5±7.4 (48-78)
Height, cm[a]	163.5±6.6 (150-180)
Weight, kg[a]	56.1±9.6 (33-79)
Performance status, 0/1/2/unknown	24/20/4/1
Differentiation, well/moderate/poor/unknown	7/28/8/6
T1/T2/T3/T4	16/6/15/12
N0/N1	22/27
M0/M1a[b]	41/8
Stage I/II/III/IVa	12/10/19/8
Clinical outcome[c]	
Complete response rate	23 (46.9%)
Grade 3/4 leucopenia	21 (42.9%)
Grade 3/4 stomatitis	7 (14.3%)
Grade 3/4 cheilitis	8 (16.3%)

These data have been previously reported (3). [a]Data are presented as the mean ± standard deviation, with the range in parentheses. [b]Non-cervical primary tumors with positive supraclavicular lymph nodes were defined as M1a. [c]Data are presented as the number of patients, with the rate in parentheses.

the patient characteristics, treatment protocol, and clinical responses in this clinical study, which was conducted with the authorization of the Ethical Committee for Genetic Studies of the Kobe University Graduate School of Medicine (Kobe, Japan) and followed the medical research council guidelines of Kobe University, have been described in our previous report (3).

Population analysis. This analysis was performed using a nonlinear mixed-effects modeling program, Phoenix® NLME™ software (v7.0; Certara USA, Inc., Princeton, NJ, USA). The first-order conditional estimation with the extended least-squares (FOCE-ELS) method was used to estimate the population parameters and their variability. Population model choice was based on Akaike's Information Criteria (AIC), goodness-of-fit plots, including observed (OBS) vs. population prediction (PRED) and vs. individual predicted concentrations (IPRED), and the coefficient of variation (CV) in parameter estimates. As a cutoff criterion for model improvement, a drop in AIC of 2 or more was applied, which is a threshold for choosing one model over another (11).

A pharmacokinetic compartment model with a cosinor method was employed to evaluate circadian variations in the plasma drug concentrations (12). However, this method is unsuitable for the evaluation of the circadian variation of the plasma 5-FU concentration in the current study because these data were obtained at only two dosing time points (05:00

and 17:00 h), which is too small a sample size to describe time-course alteration via a pharmacokinetic compartment model with a cosine curve. Therefore, population analysis using simple model equations is necessary in this case. Our research group previously found that the circadian rhythm and treatment cycle are significant factors contributing to inter- and intrapatient variations of the plasma 5-fluorouracil concentrations in patients with ESCC (3,4). Thus, in the current study, both the circadian rhythm and treatment cycle served as covariates for the model equation. To determine the final model equation, different model equations (additive, multiplicative, and power) were initially tested based on Akaike's Information Criteria (AIC), goodness-of-fit plots, and the CV of parameter estimates. In the final model, the plasma 5-fluorouracil concentration was defined using the following equation:

$$C_{5\text{-}FU} = Basis \times Circ^{kcirc} \times Cyc^{kcyc} \quad (1)$$

Where $C_{5\text{-}FU}$ is the plasma 5-FU concentration, and *Basis* is the plasma 5-FU concentration excluding the effects of the treatment cycle and circadian rhythm. *Circ* and *Cyc* are circadian rhythm and repeated treatment cycle effects on the plasma 5-FU concentration, respectively; k_{circ} and k_{cyc} are the constants governing *Circ* and *Cyc*. In the source data, the plasma 5-FU concentration at 17:00 h was higher than that at 05:00 h in the same treatment cycle; thus, to describe the effects of the circadian rhythm on the plasma 5-FU level, k_{circ} was fixed at 0 and 1.0 when the time point was 05:00 h and 17:00 h, respectively. Similarly, the plasma 5-FU concentration in the second cycle was higher than that in the first cycle at the same sampling time; therefore, to describe the effects of the treatment cycle on the plasma 5-FU level, k_{cyc} was fixed at 0 in the first cycle and at 1.0 in the second cycle. The *Basis* value means the plasma concentration of 5-FU on day 3 at 17:00 h.

The interindividual variability was described by an exponential function. The individual parameter estimate (A_i) is the product of the population parameter estimate (θ_A) and the random effect for parameter A_i (η_i), which followed a normal distribution with mean 0 and variance ω^2 and was determined using the following equation:

$$A_i = \theta_A \times e^{\eta i} \quad (2)$$

To describe the residual variability between the observed and predicted plasma concentrations (C_{obs} and C_{pred}), different error models (additive, multiplicative, and power models) were tested and chosen based on AIC and CV of the parameter estimates. The residual variability was characterized using the random effect (ε), which followed a normal distribution with mean 0 and variance σ^2, as per the following power error model:

$$C_{obs} = C_{pred} + C_{pred}^{0.5} \times \varepsilon \quad (3)$$

To perform statistical analysis on patients' characteristics and clinical outcomes, post hoc population parameter estimates for individual patients were obtained by this population analysis.

To confirm the stability of the final population model, the model was assessed by nonparametric bootstrap sampling (n=1,000). This bootstrap procedure was performed for comparison with the population model parameters estimated from the original dataset and to obtain the confidence intervals for the model parameters.

Statistical analysis. Two-group comparisons of population parameters were made by the Mann-Whitney *U* test. Comparisons across multiple groups were performed using the Kruskal-Wallis test with post-hoc comparisons by the Scheffe test. Correlation between population parameters and patients' characteristics, including clinical efficacy and toxicity, were assessed by the Pearson or Spearman rank correlation test. The patients' characteristics in the correlation test were age, height, weight, body surface area (BSA), sex, TNM staging, the clinical response, leucopenia, stomatitis, and cheilitis according to our previous report (3). P<0.05 was considered to indicate a statistically significant difference.

Results

Population analysis. Fig. 1 shows the plasma 5-FU concentration and goodness-of-fit plots of the final population model. Significant circadian variations and treatment cycle effects were observed; the plasma 5-FU concentration at 17:00 h was higher than that at 05:00 h, and that level in the second cycle was higher than that in the first cycle. However, no significant circadian variations in the second cycle were observed, possibly due to large interindividual variability in the second cycle. The goodness-of-fit plots indicated that the population model with a random effect adequately described the individual predictions of the plasma 5-FU concentration. The population parameter estimates and the results of the bootstrap validation are described in Table II. These parameter and random effects were estimated with relatively high precision because all were <39.8% and were very similar to the mean of the bootstrap procedure. Population analysis with the covariates of circadian variations and repeated treatment cycle effects revealed that the basis plasma 5-FU concentration without the covariates (*Basis*) was 0.078 μg/ml at the steady state during the prolonged infusion of 5-FU at 400 mg/(m²·day), with relatively low inter- and intraindividual variability (13.5 and 24.2%, respectively). The population parameters indicated that the plasma 5-FU concentration was 1.3-fold higher in the evening than in the morning, and that level in the second cycle was 1.5-fold greater than that in the first cycle.

Population parameters and clinical responses. The population parameters of the patients with a survival period of 5 years or more and with less than 5 years are shown in Table III, and for the patients with a complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD), the parameters are given in Table IV. No significant differences between survival time groups were observed in any population parameters. However, significant differences (in the population parameters) were observed in therapeutic efficacy, with higher *Basis* values in patients with CR than in those with PR (P=0.034). *Cyc* values in patients with CR were slightly higher
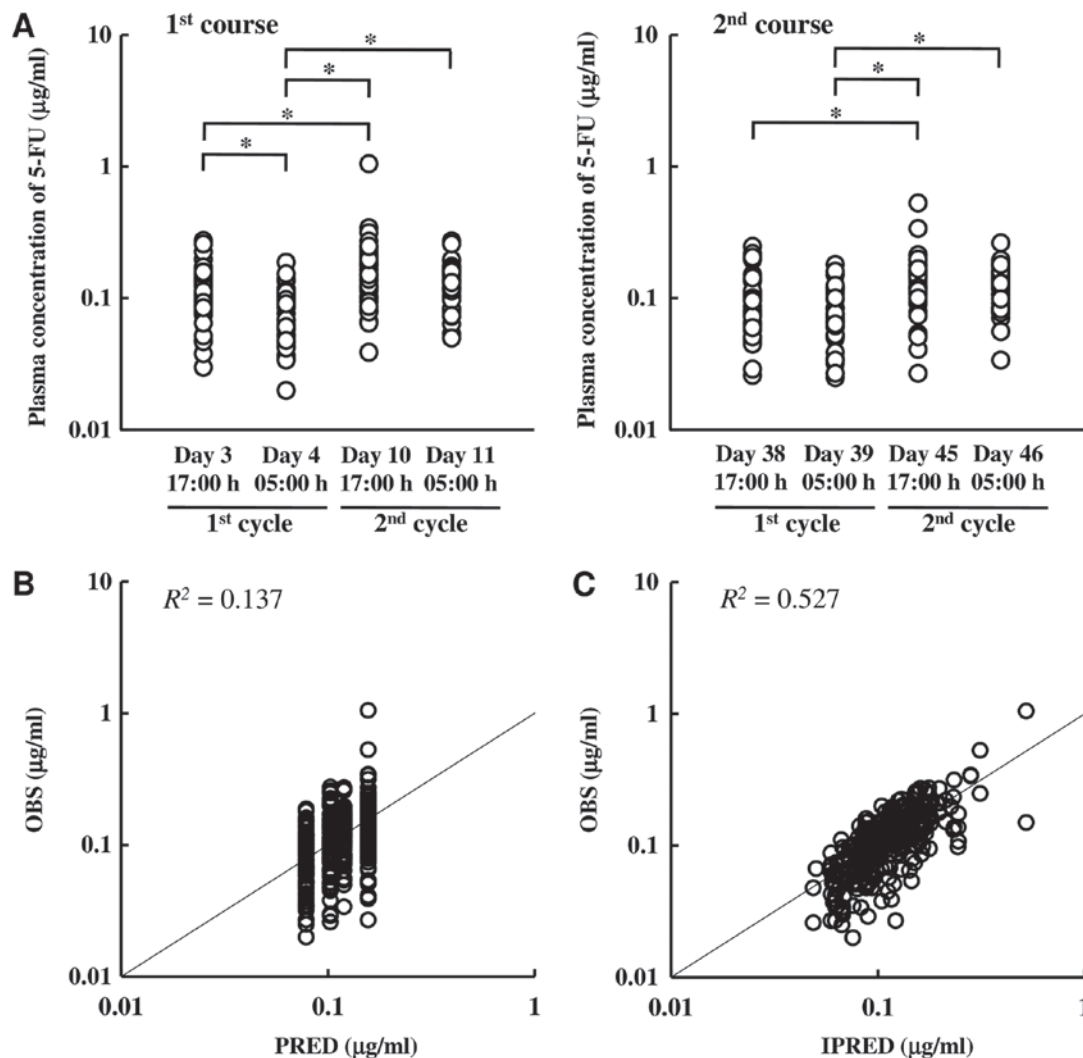
Figure 1. Circadian and chemotherapeutic cycle effects on the plasma concentration of 5-fluorouracil (5-FU) and population model diagnostic plots. (A) The plasma concentration of 5-FU after definitive 5-fluorouracil/cisplatin-based chemoradiotherapy in patients with ESCC; (B) goodness-of-fit plots for the final population model, the observed 5-FU plasma concentrations (OBS) vs. population model predictions (PRED); (C) OBS vs. individual model predictions (IPRED); *P<0.05: A statistically significant difference according to the Kruskal Wallis test with post-hoc comparisons by the Scheffe test. ESCC, esophageal squamous cell carcinoma.

than those in patients with PR, although the different was not significant (P=0.062).

Fig. 2 presents a significant linear correlation between population parameters and patients' characteristics. There were significant correlations in *Basis* vs. age and height, whereas no correlations were observed with BSA, sex, or TNM staging. Clinical responses (CR, PR, SD, and PD) significantly correlated with *Basis* values (P=0.025). The grade of cheilitis after the second course also varied according to *Basis* values. A significant negative correlation was observed between *Cyc* and leucocyte counts obtained before the start of chemoradiotherapy. Stomatitis and cheilitis after the second course also correlated with *Cyc* values. There were no significant correlations between *Circ* values and patients' characteristics.

Discussion

In the field of oncology, researchers are focusing on the TDM of 5-FU, which improves the clinical outcome. However, circadian variation and repeated treatment cycle effects on the plasma concentration of 5-FU exist as confounding factors in the determination of the appropriate blood sampling time and plasma 5-FU level. Unfortunately, the correlations between the size of these effects on plasma 5-FU concentration and the clinical response, including long-term survival and toxicities, are still unknown. In the current study, to evaluate the circadian rhythm and treatment cycle effects on the plasma 5-FU concentration, population analysis was performed using previously reported data on patients with ESCC after definitive 5-FU/CDDP-based chemoradiotherapy, and correlations with clinical responses were investigated.

The population analysis revealed that the estimated *Basis* parameter, which shows the plasma concentration of 5-FU on day 3 at 17:00 h, significantly correlated with the clinical response (CR, PR, PD, and SD), and higher values were observed in patients with CR, whereas there was no correlation with survival time after chemoradiotherapy. Although the 5-FU dose was determined based on BSA, there was no correlation between the plasma 5-FU level and BSA; older and shorter patients had higher estimated *Basis*. Clinical studies

Table II. Population parameters of 5-FU regarding circadian and repeated treatment cycle effects in Japanese patients with ESCC.

Parameters	Unit	Final model		Bootstrap (n=1,000)		
		Estimate	CV, %	Mean	Median	2.5th-97.5th percentiles
Fixed effect parameters						
Basis	μg/ml	0.078	5.4	0.079	0.079	0.070-0.088
Circ		1.308	5.4	1.31	1.30	1.183-1.460
Cyc		1.522	4.9	1.52	1.51	1.369-1.699
Inter-individual variability						
ω (*Basis*)	%	24.2	26.2	23.7	23.7	18.595-28.288
ω (*Circ*)	%	23.3	39.8	22.6	22.3	0.237-33.038
ω (*Cyc*)	%	16.8	36.0	16.1	16.2	0.087-25.068
Residual variability						
σ	%	13.5	4.5	13.4	13.3	11.086-16.434

ESCC, esophageal squamous cell carcinoma; *Basis*, plasma 5-FU concentration excluding the effects of treatment cycle and circadian rhythm; *Circ*, the effects of circadian rhythm on plasma 5-FU concentration; CV, coefficient of variation; *Cyc*, the effects of treatment cycle on plasma 5-FU concentration; 5-FU, 5-fluorouracil.

Table III. Association between prognosis after treatment with a definitive 5-FU/cisplatin-based chemoradiotherapy and population parameters of 5-FU regarding treatment cycle and circadian variation in 49 Japanese patients with ESCC.

Parameters	Total (n=49)	Survival of ≥5 years (n=21)	Survival of <5 years (n=28)	P-value[a]
Basis, μg/ml	0.079±0.015	0.081±0.016	0.077±0.014	0.480
Circ	1.305±0.205	1.346±0.277	1.274±0.123	0.657
Cyc	1.525±0.138	1.534±0.169	1.519±0.113	0.952

[a]Survival of ≥5 years or more vs. <5 years by Mann-Whitney's U test. Data are presented as the mean ± standard deviation. ESCC, esophageal squamous cell carcinoma; *Basis*, plasma 5-FU concentration excluding the effects of treatment cycle and circadian rhythm; *Circ*, the effects of circadian rhythm on plasma 5-FU concentration; *Cyc*, the effects of treatment cycle on plasma 5-FU concentration; 5-FU, 5-fluorouracil.

Table IV. Association between clinical outcome after treatment with a definitive 5-FU/cisplatin-based chemoradiotherapy and population parameters of 5-FU regarding treatment cycle and circadian variation in 49 Japanese patients with ESCC.

Parameters	CR (n=23)	PR (n=21)	SD (n=2)	PD (n=2)	P-value[a]
Basis, μg/ml	0.084±0.016	0.075±0.011	0.062, 0.067	0.061, 0.095	0.034
Circ	1.312±0.257	1.305±0.163	1.324, 1.339	1.263, 1.310	0.751
Cyc	1.564±0.163	1.489±0.104	1.529, 1.682	1.454, 1.476	0.062

[a]CR vs. PR by Mann-Whitney's U test. Data for CR and PR are presented as the mean ± standard deviation, and individual patient data for SD and PD. ESCC, esophageal squamous cell carcinoma; *Basis*, plasma 5-FU concentration excluding the effects of treatment cycle and circadian rhythm; *Circ*, the effects of circadian rhythm on plasma 5-FU concentration; *Cyc*, the effects of treatment cycle on plasma 5-FU concentration; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; 5-FU, 5-fluorouracil.

have shown that in the FOLFIRI and FOLFOX regimens for the treatment of colorectal cancer, pharmacokinetic-guided dose adjustment of 5-FU can enhance and improve the clinical efficacy as compared to BSA-based dosage (1,2). Our results suggest that, in agreement with the regimens for colorectal cancer, in the definitive 5-FU/CDDP-based chemoradiotherapy for the treatment of ESCC, dose adjustment based on the plasma 5-FU level could be a valuable approach to improving clinical responses. The results of the current study also suggest that the appropriate blood sampling time for estimating the clinical response is the evening of day 3. Dose management based on the patient's age and height with monitoring of the plasma 5-FU level on the evening of day 3 may be an effective approach to improving clinical efficacy in patients with ESCC after the definitive 5-FU/CDDP-based chemoradiotherapy. However, the number of sampling time
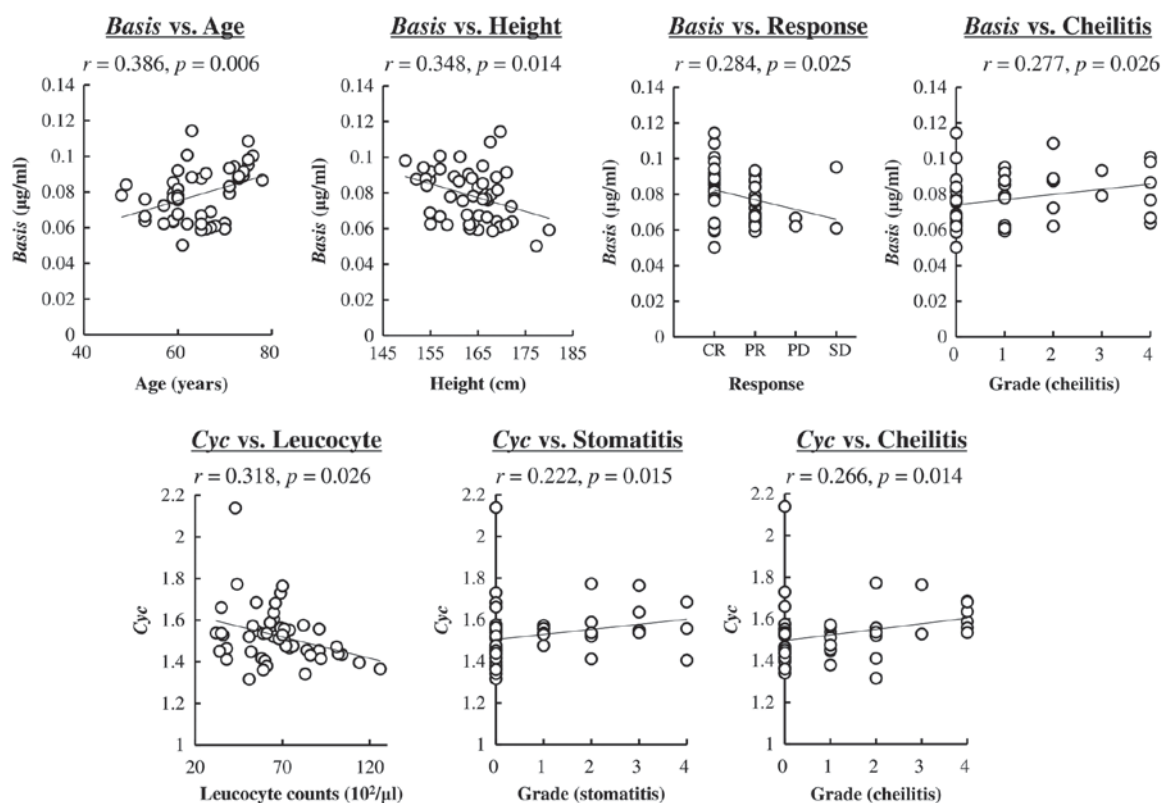
Figure 2. A linear relation between individual population parameters and patients' characteristics including the clinical response after definitive 5-fluorouracil/cisplatin-based chemoradiotherapy in patients with ESCC. The cheilitis and stomatitis data were obtained after the second course of treatments. The leucocyte counts were determined before the start of the chemoradiotherapy. *Cyc*, the effects of treatment cycle on plasma 5-FU concentration; ESCC, esophageal squamous cell carcinoma.

points within the day was small in the current study; further studies are necessary to determine the optimal blood sampling time.

A subsequent cycle of treatment was estimated to lead to a 1.5-fold greater plasma 5-FU level. Of note, *Cyc* significantly correlated with pretherapeutic leucocyte counts: Lower leucocyte counts had greater effects on the magnitude of plasma 5-FU level elevation in the second cycle. Higher 5-FU concentrations in the plasma after repeated treatment with 5-FU in animals and patients have been reported (4,5,13,14). This 5-FU elevation is related to a decrease in clearance and hepatic dihydropyrimidine dehydrogenase (DPD) activity levels (13,14). Repeated 5-FU administration also leads to the loss of the circadian rhythm in hepatic DPD activity (4,13). The plasma ratio of dihydrouracil/uracil (UH_2/Ura) is known to be an indirect response marker of DPD activity in the liver and peripheral blood mononuclear cells (15), and our previous study using a rat model of colorectal cancer found that this ratio can assess the higher AUC of 5-FU and lower DPD activity during repeated 5-FU administration (14). Nevertheless, the measurement of dihydrouracil and uracil concentration in plasma needs highly sensitive analysis using high-performance liquid chromatography or liquid chromatography with tandem mass spectrometry, which require much more experimental time and is expensive. Therefore, according to the present results, leucocyte counts obtained by the routine diagnostic test before the definitive 5-FU/CDDP-based chemoradiotherapy could be more valuable for estimating the elevation in plasma

5-FU concentration during a subsequent cycle of treatment. However, the mechanism of their correlation is still unknown and further studies are needed.

The analysis in the current study detected a correlation between the plasma concentration of 5-FU and its toxicity. The occurrence of cheilitis and stomatitis after the second course of the definitive 5-FU/CDDP-based chemoradiotherapy was affected by the plasma concentration of 5-FU on day 3 at 17:00 h (parameter *Basis*) and the elevation in plasma 5-FU concentration during a subsequent treatment cycle (parameter *Cyc*). Some clinical studies have revealed that pharmacokinetics-guided dose adjustment of 5-FU can reduce the toxicity (2,16,17). Thus, the plasma 5-FU concentration on the evening of day 3 and pretherapeutic leucocyte counts could be important markers for evaluating both clinical efficacy and toxicity.

Our data showed that circadian variations in the plasma concentration of 5-FU during continuous infusion were relatively small and did not contribute to the clinical response or toxicity, in line with the previous report (5). Fleming *et al* (5), raised the possibility that the benefit from chronomodulated chemotherapy with 5-FU observed in clinical studies is related to proliferation and/or drug metabolic rhythms in tumor tissue, not circadian variations in the plasma 5-FU level. It has been shown that there is significant circadian variation in cell proliferation (18-21). Results of population PK-PD model analysis in rats show that not only fluctuations in plasma 5-FU concentration but also the cell sensitivity to 5-FU affect the onset and severity of its toxicity (22). The modulation of the

infusion rate throughout the day according to cell proliferation may improve the clinical response to 5-FU/CDDP-based chemoradiotherapy.

The results of the current study show a relation between the factors affecting plasma 5-FU concentration and the clinical response in patients with ESCC after treatment with the definitive 5-FU/CDDP-based chemoradiotherapy. To obtain a better clinical response to this chemoradiotherapy, the measurement of plasma 5-FU concentration on the evening of day 3 and leucocyte counts before chemotherapy may represent a good therapeutic strategy for the definitive 5-FU/CDDP-based chemoradiotherapy because it would help to predict the clinical response. This measurement of the plasma component may aid the decision on whether to continue chemotherapy to sequential cycles. However, this study has some limitations. First, we evaluated only two blood sampling time points (5:00 h or 17:00 h) throughout the day and furthermore, the sample size was small. Second, it is difficult to exclude radiation effects from our original data to analyze the circadian rhythm chemotherapy. The radiation may be one of covariates for plasma concentrations of 5-FU (23). However, in the current study, all patients have been treated with 2 Gy/day on days 1-5 and then covariate analysis of radiation could not be performed in the current original data set. Therefore, the results of this study could apply to only the definitive 5-FU/CDDP-based chemoradiotherapeutic regimen, not the other 5-FU-bsed regimen. Finally, the current analysis was retrospective, i.e., using previously reported data. To demonstrate the usefulness of measuring the plasma concentration of 5-FU and leucocyte counts before chemotherapy, we suggest a prospective study with a larger sample size.

In conclusion, the findings of the present study provide evidence that plasma concentration of 5-FU, excluding the circadian and repeated treatment cycle effects, depends on age, height, and the clinical response in patients with ESCC after treatment with the definitive 5-FU/CDDP-based chemoradiotherapy. The circadian variations in plasma 5-FU concentration were relatively small, and the magnitude of the repeated cycle effect correlated with leucocyte counts before chemotherapy. These results suggest that the initial dosage of 5-FU based on age and height may offer a higher plasma concentration of 5-FU and clinical response. The measurement of leucocyte counts before the start of chemoradiotherapy may estimate the increase in the plasma 5-FU level after a sequential cycle and help to avoid severe toxicity. To clarify the advantages of these proposed chemoradiotherapy regimens, additional prospective studies with a larger sample size are needed.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

AK and SK analyzed the clinical data and wrote the manuscript. TT designed the study and was a major contributor to writing of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The source of data was obtained from the previous reported study that was conducted with the authorization of the Ethical Committee for Genetic Studies of the Kobe University Graduate School of Medicine (Kobe, Japan) and followed the medical research council guidelines of Kobe University, Japan. Informed consent was obtained from all individual participants included in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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