Regular Article

Changes in Pharmacodynamic Parameters during Co-administration of 5-FU with Warfarin: A Retrospective Case Series

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Drug–drug interactions (DDIs) between warfarin (WF) and fluoropyrimidines are well known. Co-administration of WF and 5-fluorouracil (5-FU) leads to elevations in prothrombin time international normalised ratio (PT-INR). The inhibition of drug metabolism through suppression of CYP activity is a possible cause of prolonged PT-INR elevations. 5-FU and its metabolites are suspected to inhibit CYPs, but the precise mechanisms of action remain unknown. This study aimed to investigate the possible DDI effects of the co-administration of 5-FU with WF using PT-INR and PT-INR/dose ratio as pharmacodynamic parameters. Retrospective case series data were collected from patients who received parenteral 5-FU chemotherapy from April 2009 to December 2019 at the University of the Ryukyus Hospital. Seven patients who received 5-FU in combination with WF were analysed. There was a significant increase in PT-INR and PT-INR/dose during the co-administration of WF and 5-FU ($p = 0.0018$ and $p = 0.0187$, respectively; paired *t*-test). The findings demonstrated significant DDI between 5-FU and WF evident as elevated PT-INR and PT-INR/dose ratio.

Key words 5-fluorouracil; warfarin; fluoropyrimidine; CYP; drug interaction; chemotherapy

INTRODUCTION

Many cancer patients require anticoagulation therapy due to the high risk of cancer-associated thromboembolic events or pre-existing comorbidities, such as atrial fibrillation and heart valve replacement. These overlaps in anti-cancer and anticoagulation therapies are increasingly encountered in aging populations.[1)]

Although known for its narrow therapeutic index, the vitamin K antagonist warfarin (WF) remains the most common anticoagulant prescribed for deep vein thrombosis, pulmonary embolism, atrial fibrillation, and heart valve replacement, as well as for thromboprophylaxis in patients with cancer.[1–3)] WF consists of a racemic mixture of S-WF and R-WF, which are mainly metabolised by CYP2C9 and 3A4, respectively.[4)]

Regimens based on the fluoropyrimidine 5-fluorouracil (5-FU) have been the mainstay of chemotherapy for malignancies that include colorectal,[5–13)] breast,[6–12)] head and neck,[8,12)] and gastric cancers.[7,11–13)] Several reports in the literature have described possible drug–drug interactions (DDIs) between WF and fluoropyrimidines,[7–11,13–15)] but the majority of these studies involved oral fluoropyrimidines. Studies documenting 5-FU and WF DDIs discussed the possibility that CYP2C9 inhibition is the main pathway for elevated prothrombin time international normalised ratio (PT-INR) during co-administration.[8,10,14–16)] 5-FU or its metabolites are suspected to inhibit CYP2C9 and CYP3A4. However, the exact mechanisms of 5-FU inhibition of CYPs remain unknown.[6,7,9,11,13,17)] 5-FU does not directly inhibit CYP enzymes.[18)] In our previous *in vitro* study, HepaRG cells exposed to 5-FU at 10 μg/mL for 96 h showed significant reduction in mRNA levels of CYP3A4 and NR1I2 (a regulator of CYPs), but not in CYP2C9 mRNA.[17)] The active metabolites of 5-FU inhibit DNA synthesis, cause DNA damage, disrupt RNA processing, and alter normal RNA function.[19–25)] Taken together, these results suggest the possibility that 5-FU suppresses CYP protein expression by inhibiting its transcriptional regulation. Additionally, since R-WF has a longer half-life[6)] and is also a non-competitive inhibitor of CYP2C9,[26)] the clinical involvement of CYP3A4 may be more significant, wherein the effects of 5-FU co-administration become apparent after at least a few weeks.[6,7,9,11)] We hypothesised that the duration of most *in vitro* studies performed to date might have been too short to reflect the conditions necessary for the onset of pharmacodynamic (PD) changes attributable to 5-FU and WF DDIs in clinical settings. Since there are few studies on bolus or intravenous infusion of 5-FU, this retrospective case series investigated the long-term DDIs of WF and parenteral 5-FU, in which larger doses of 5-FU were administered compared to oral administration, using the PT-INR and PT-INR/dose ratio as PD parameters.

MATERIALS AND METHODS

Study Design In this retrospective case series, we collected data from the electronic medical records of patients who received parenteral 5-FU chemotherapy from April 2009 to December 2019 at the University of the Ryukyus Hospital. We identified all the patients who received 5-FU chemotherapy in combination with WF. Patient information that was reviewed and analysed included sex, age, height, weight, body surface area, length of chemotherapy cycle, indication for 5-FU, che-

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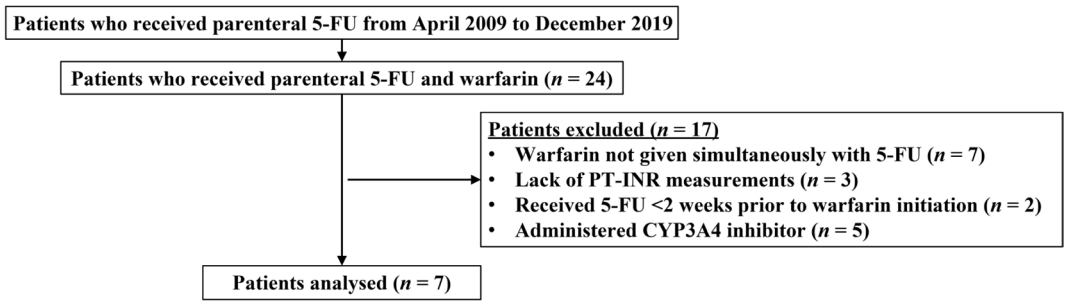

Fig. 1. Study Design and Flowchart for Eligible Patients

5-FU, 5-fluorouracil; PT-INR, prothrombin time international normalised ratio.

motherapy regimen, dose of 5-FU, indication for WF, dose of WF, PT-INR, and laboratory indicators of hepatic and renal function. The WF titre was evaluated using the PT-INR/dose ratio.[13] Since there were patients who received multiple cycles of chemotherapy during the study period, we limited the patients according to the following criteria: to account for a washout period of 14 d,[14] only patients who had no 5-FU exposure for at least 2 weeks prior to WF initiation were included, and only patients who did not receive any known CYP3A4 or 2C9 inhibitors were analysed.

Ethical Considerations This study was conducted in accordance with the principles of the Declaration of Helsinki, in compliance with the "Ethical Guidelines for Medical and Health Research Involving Human Subjects," and with the approval of the University of the Ryukyus Ethics Review Committee (Approval No. 1102).

Statistical Analyses Data are expressed as mean ± standard deviation (S.D.) unless otherwise stated. Changes following co-administration of 5-FU in hepatic and renal function indicators, PT-INR, WF daily dosage, and PT-INR/dose were analysed by paired *t*-test. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using GraphPad Prism version 8.0.2 for Windows (GraphPad Software, San Diego, CA, U.S.A.).

RESULTS

Patient Characteristics The study design and flowchart for identifying patients eligible for the analysis are shown in Fig. 1. We identified 24 patients who received parenteral 5-FU chemotherapy in combination with WF from April 2009 to December 2019 at the University of the Ryukyus Hospital. Twelve patients were excluded because WF was initiated only after receiving 5-FU, PT-INR measurements were lacking, and 5-FU exposure was <2 weeks before WF initiation. Five patients were excluded because they had been administered CYP3A4 inhibitors. Seven patients were eligible for the analysis.

Table 1 summarises the characteristics of the seven patients. Hepatic and renal function tests did not show significant changes from baseline values before chemotherapy to those during 5-FU administration (Table 2).

Changes in WF Daily Dose, PT-INR, and PT-INR/Dose Two cases showed the attending physician's awareness of 5-FU and WF DDIs, as indicated in the records, when they ordered WF dose adjustments or a shift to intravenous heparin prior to the start of chemotherapy. There were no bleeding complications, adverse events, or indications for rescue therapy in any of the patients.

Table 1. Baseline Patient Characteristics

Characteristics	Total patients ($n = 7$)
Male/Female (*n*)	4/3
Age (years)	61 ± 12
Height (cm)	161 ± 8
Weight (kg)	58 ± 10
BSA (m^2)	1.60 ± 0.15
Chemotherapy cycle (*n*)	
14-d cycle	4
28-d cycle	3
5-FU (mg/d)	
Mean ± S.D.	2014 ± 1175
Median	1448 (534–3550)
Warfarin daily dose (mg/d) (Pre-Chemo)	
Mean ± S.D.	2.6 ± 1.4
Median	2.0 (1.5–5.0)
5-FU Indication (*n*)	
Colorectal cancer	3
Head and neck cancer	3
Oesophageal cancer	1
Chemotherapy regimens (*n*)[a]	
NDP + 5-FU	3
Biweekly DCF	1
mFOLFOX-6 pump	1
FOLFIRI + Pmab	1
FOLFIRI + BV (5)	2
FOLFIRI + Cmab (biweekly)	1
mFOLFOX-6 + Pmab	1
Warfarin indication (*n*)[b]	
Atrial fibrillation	3
Deep vein thrombosis	1
Aortic valve replacement	1
Pulmonary artery thrombosis	1
Superior vena cava thrombosis	1
Superior vena cava syndrome	1

S.D., standard deviation; BSA, body surface area; NDP, nedaplatin; DCF, docetaxel, cisplatin, and 5-fluorouracil; FOLFOX-6, leucovorin calcium (folinic acid), fluorouracil, and oxaliplatin; FOLFIRI, leucovorin calcium (folinic acid), fluorouracil, irinotecan hydrochloride; Pmab, panitumumab; BV, bevacizumab; Cmab, cetuximab. *a*) Some patients received multiple regimens. *b*) Some patients were administered warfarin for multiple indications.

The changes in the WF daily dose, PT-INR, and PT-INR/dose are presented in Fig. 2. The changes in the baseline WF daily dose before chemotherapy were compared with the minimum dose administered within a chemotherapy cycle. As expected, the mean daily dose of WF decreased during che-

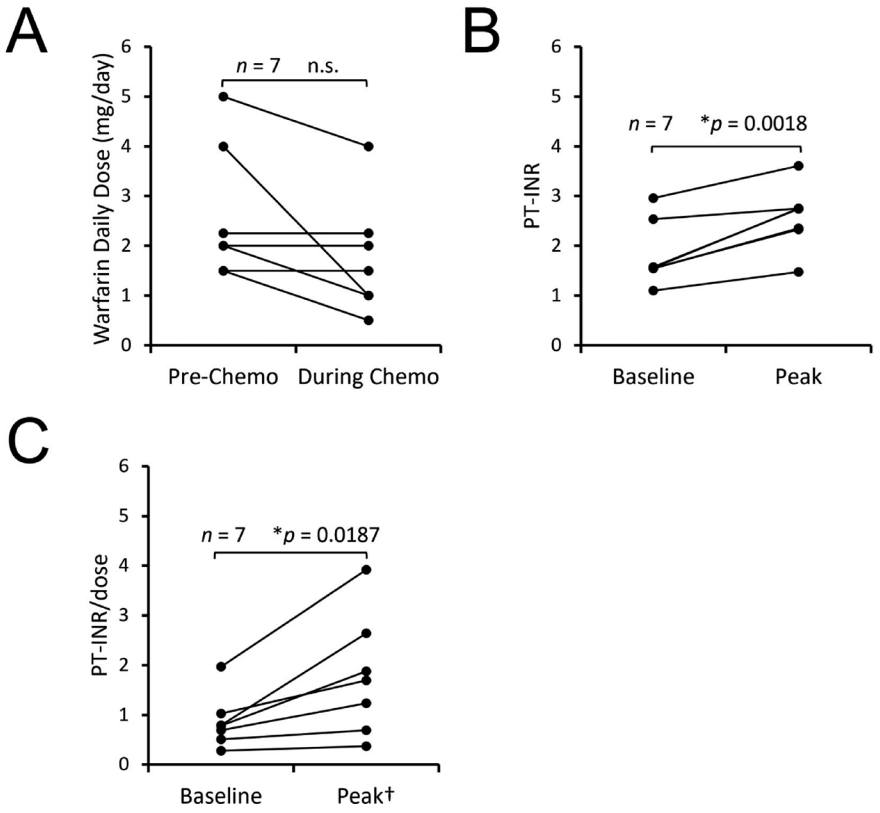

Fig. 2. Changes in Warfarin Daily Dose, PT-INR, and PT-INR/Dose

Patients shown individually (paired *t*-test). (A) Changes in warfarin daily dose before and during chemotherapy. *Pre-Chemo* represents the dosage of warfarin given on or closest to Day 0 of chemotherapy. *During Chemo* represents the minimum dosage of warfarin given within a chemotherapy cycle. (B) Changes in PT-INR (there are overlaps). *Baseline* represents the PT-INR reported on or closest to Day 0 of chemotherapy. *Peak* represents the highest PT-INR reported within a chemotherapy cycle. (C) Changes in PT-INR/dose. *Baseline* represents the PT-INR/dose on or closest to Day 0 of chemotherapy. *Peak*† represents the *highest PT-INR/dose, regardless of corresponding warfarin daily dose*, either within a chemotherapy cycle or after the last day of the cycle. Chemotherapy cycle (Day 1 to Day 14 or 28); n.s., not statistically significant.

Table 2. Hepatic and Renal Function Tests

Function test (Mean ± S.D.)	Total patients ($n = 7$)	
	Pre-chemo	During chemo
AST (IU/L)	22.14 ± 11.68	26.71 ± 10.97
ALT (IU/L)	22.43 ± 15.61	26.14 ± 18.32
Crea (mg/dL)	0.62 ± 0.19	0.58 ± 0.17
eGFR (mL/min/1.73 m^2)	98.50 ± 18.94	101.80 ± 28.23

S.D., standard deviation; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Crea, creatinine; eGFR, estimated glomerular filtration rate. Changes from pre-chemotherapy to during chemotherapy values were not statistically significant (paired *t*-test).

Table 3. Changes in Warfarin Parameters after 5-FU Co-administration

Parameter	Total patients ($n = 7$)		*p*-Value
	Pre-chemo	During chemo	
Warfarin daily dose (mg/d)			
Mean ± S.D.	2.61 ± 1.35	1.75 ± 1.16	n.s.
Median	2.00 (1.50–5.00)	1.50 (0.50–4.00)	
PT-INR			
Mean ± S.D.	1.83 ± 0.66	2.57 ± 0.64	0.0018
Median	1.56 (1.10–2.96)	2.74 (1.47–3.61)	
PT-INR/dose[a)]			
Mean ± S.D.	0.86 ± 0.54	1.77 ± 1.21	0.0187
Median	0.78 (0.28–1.97)	1.69 (0.37–3.92)	

S.D., standard deviation; PT-INR, prothrombin time international normalised ratio; n.s., not significant. Changes within groups pre-chemotherapy and during chemotherapy were compared by paired *t*-test. *a*) Changes from pre-chemotherapy to during or after chemotherapy values were compared by paired *t*-test.

motherapy, specifically by 33% of the pre-chemotherapy dose. This change was not statistically significant (Table 3). There were statistically significant increases in PT-INR (by 40% of the pre-chemotherapy value) and PT-INR/dose (by 105% of the pre-chemotherapy value) from baseline to peak levels upon co-administration of WF with 5-FU. The mean number of days to peak PT-INR, to peak PT-INR/dose, of the 5-FU and WF co-administration/chemotherapy cycle, and to the first WF dose adjustment after the end of co-administration are presented in Table 4. After completion of chemotherapy, and with continuous administration of WF, the elevated PT-INR and PT-INR/dose returned to baseline values (Supplementary Materials).

DISCUSSION

We investigated the DDIs of WF following long-term exposure to 5-FU using PD parameters, which has not been investigated in *in vitro* studies. To the best of our knowledge, this is the first study to evaluate the PT-INR/dose of WF as a PD parameter in patients co-administered parenteral 5-FU and WF. After co-administration with 5-FU, there was a decrease in the daily dose of WF. However, during the same period, PT-INR was significantly increased. Therefore, we also anal-

Table 4. Time Course of Events after 5-FU Co-administration

Event	Total patients ($n = 7$)
Days to peak PT-INR	
Mean ± S.D.	14.1 ± 10.6
Median	15.0 (2.0–27.0)
Days to peak PT-INR/dose	
Mean ± S.D.	20.0 ± 10.9
Median	25.0 (2.0–30.0)
Days of 5-FU & WF co-administration/chemo cycle	
Mean ± S.D.	3.3 ± 2.1
Median	5.0 (1.0–5.0)
Days after end of co-administration to first WF dose adjustment	
Mean ± S.D.	10.6 ± 11.7
Median	2.0 (0.0–25.0)

S.D., standard deviation; PT-INR, prothrombin time international normalised ratio.

ysed the changes in PT-INR/dose, in addition to the changes in WF daily dose and PT-INR. PT-INR/dose was considered a PD parameter to evaluate DDI because it can simultaneously reflect the changes due to inhibition of drug metabolism, such as the decrease in drug dosage and the increase in PT-INR. The significant increase in PT-INR/dose from baseline to peak levels during 5-FU co-administration suggested a significant interaction between 5-FU and WF (which is metabolised by CYP2C9 and 3A4). A case report on the concomitant use of WF with doxifluridine[27] and a retrospective case series comparing co-administered WF + S-1 with WF + capecitabine reported similar increases in PT-INR/dose.[13] Moreover, these changes were reversible after completion of 5-FU administration, suggesting a DDI with WF. Similar reversible changes in PT-INR were reported in patients who received WF with S-1 and capecitabine.[14]

The available evidence so far suggests that the molecular mechanisms behind 5-FU and CYP interactions might be due to 5-FU's inhibition of DNA synthesis and interference in normal RNA function.[19–25] In our previous study using HepaRG cells to investigate 5-FU inhibition of pharmacokinetic-related gene expression, CYP3A4 mRNA levels were significantly reduced after exposure to 10 μg/mL 5-FU for 96 h.[17] CYP2C9 mRNA levels were not affected, but mRNA levels of NR1I2 (a regulator of CYPs) were also significantly reduced.[17] In contrast, the levels of CYP2C6 and CYP2C11 mRNA in rats, the functional counterparts of human CYP2C9, treated with 5-FU (120 mg/kg, single intraperitoneal injection) were significantly decreased.[28] The study used qRT-PCR using RNA extracted from rat livers 4 d after 5-FU administration.[28] In a follow-up study using CYP3A1/3A3 and CYP3A2, the functional counterparts of human CYP3A4 and 3A5, Fukuno *et al.* reported that liver microsomes from rats (extracted 2 d after 5-FU administration) treated with the same dose of 5-FU showed significantly increased protein levels of CYP3A2, but not CYP3A1/3A3, by Western blot analysis.[29] However, along with the increased protein levels, the authors observed a concurrent decrease in midazolam affinity for CYP3A.[29] Fukuno *et al.* hypothesised that 5-FU inhibits CYP3A neither by competitive nor mechanism-based inhibition but through post-transcriptional modification.[29] Similarly, the authors hypothesised that 5-FU activity decreased mRNA expression of *CYP2C6* and *CYP2C11*, suggesting the suppression of CYP2C transcription and/or enhancement of mRNA degradation.[28] The conflicting results could possibly reflect the differences between human and rat CYPs, and an earlier study in human liver microsomes reported that 5-FU showed no significant inhibitory effect on CYP-catalysing activities.[18] Previous clinical studies have noted that the effects of 5-FU and WF DDIs appear after at least a few weeks.[6,7,9,11] In our study, the mean times to peak PT-INR and peak PT-INR/dose were at least 2 and 3 weeks, respectively (Table 4). These findings were consistent with those of previous clinical studies. The turnover half-lives of human hepatic CYPs have been estimated to be 104 h for 2C9 and 44–140 h for 3A4.[30] A period of 2–3 weeks is required for normally functioning CYP enzymes to be completely replaced by 5-FU altered enzymes. Additionally, 5-FU suppression of CYP mRNA transcription may inhibit *de novo* enzyme synthesis, thereby further delaying the elimination of CYP substrates. These findings may explain the longer time needed for 5-FU DDI effects to manifest clinically. After the end of 5-FU co-administration, it took approximately 11 d until the first WF dose adjustment, which is close to the period when 5-FU effects on PT-INR may begin to appear. This may also explain why the DDI effects persist for weeks after 5-FU administration is terminated.[11,14,27] Post-transcriptional modification of CYP proteins by 5-FU would similarly require 2–3 weeks for the altered enzymes to be completely degraded. Even with the shortest half-life estimate of 44 h for CYP3A4,[30] which would require at least 10 d for complete enzyme turnover, R-WF has a half-life of 37.4–88.6 h and may continue to affect S-WF metabolism through non-competitive inhibition of CYP2C9.[6] Although there was a significant increase in PT-INR suggesting DDI between 5-FU and WF, the time to peak PT-INR was around 20 d. Previous *in vitro* studies have not investigated long-term exposure to 5-FU. Therefore, the clinical data obtained in this study should be confirmed by further studies investigating the mechanisms involved in long-term 5-FU exposure. The significant increase in the peak PT-INR/dose also strongly suggests that the mechanism of DDI is through 5-FU inhibition of WF metabolism.

Limitations that should be taken into consideration include the small sample size, discontinuity in WF therapy (particularly for outpatients), and physician awareness of 5-FU and WF DDIs and corresponding interventions.

This study shows that the co-administration of parenteral 5-FU and WF led to significant elevations in PT-INR and PT-INR/dose. These elevations in PT-INR and PT-INR/dose may also occur in regimens that use oral fluoropyrimidines, such as capecitabine or S-1. However, the effects of 5-FU and WF DDIs require a longer time to appear clinically compared with the time observed in *in vitro* studies. Therefore, close monitoring of coagulation parameters and careful management are necessary while always considering the possibility of adverse events.

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials This article contains supplementary materials.

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