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Effects of 5-fluorouracil co-administration on blood pressure in patients maintained on antihypertensives: a retrospective case series

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This study aimed to investigate the possible drug-drug interactions (DDIs) of 5-FU with antihypertensives metabolised by CYP3A4 and 2C9, using blood pressure (BP) as a pharmacodynamic (PD) parameter. Patients who received 5-FU in combination with antihypertensives metabolised by CYP3A4 or 2C9, specifically, a) amlodipine, nifedipine, or amlodipine + nifedipine, b) candesartan or valsartan, or c) amlodipine + candesartan, amlodipine + losartan, or nifedipine + valsartan, (Group A, n = 20) were identified. Patients who received 5-FU with WF and antihypertensives, specifically, a) amlodipine or b) amlodipine + telmisartan, amlodipine + candesartan, or amlodipine + valsartan, (Group B, n = 5) or 5-FU alone (Group C, n = 25) were also identified and analysed as a comparator and control group, respectively. Regarding the peak BP levels during chemotherapy, there was a significant increase in both SBP (P < 0.0002 and 0.0013) and DBP (P = 0.0243 and 0.0032) in Groups A and C, respectively (Tukey-Kramer test). In contrast, although SBP also increased in Group B during chemotherapy, the change was not statistically significant and there was a decrease in DBP. The significant increase in SBP can be attributed to chemotherapy-induced hypertension by 5-FU or other drugs in the chemotherapeutic regimens. However, when comparing the lowest BP levels during chemotherapy, there was a decrease in SBP and DBP in all groups from the baseline values. The median time to peak and lowest BP was at least 2 weeks and 3 weeks, respectively, for all groups, suggesting that a BP lowering effect was observed following the offset of the initial chemotherapy-induced hypertension. At least 1 month after 5-FU chemotherapy, the SBP and DBP returned to baseline values in all groups. Since Group B also showed a significant increase in PT-INR, possibly demonstrating 5-FU inhibition of CYP activity and, consequently, of WF metabolism, it is likely that 5-FU also inhibited the metabolism of the antihypertensive drugs. The findings suggest possible DDIs between 5-FU and antihypertensives metabolised by CYP3A4.

1. Introduction

Co-administration of anti-cancer and anticoagulation therapies is common in clinical practice (Rose et al. 2007). Warfarin (WF), despite its inconveniences, is still often used in the treatment of atrial fibrillation, pulmonary embolism, and deep vein thrombosis, and it is also used in post-surgical care after heart valve replacement and in cancer thromboprophylaxis (Brunetti et al. 2017; Fanola et al. 2018; Rose et al. 2007). The racemic mixture of WF consists of S-WF, metabolised mainly by Cytochrome P450 (CYP) 2C9, and R-WF, metabolised by CYP3A4 (Jones et al. 2010).

5-Fluorouracil (5-FU) and other fluoropyrimidines have been the mainline chemotherapeutic agents in the treatment of head and neck (Rich et al. 2004; Shah SR et al. 2010), breast (Camidge et al. 2005; Copur et al. 2001; Kolesar et al. 1999; Rich et al. 2004; Saif 2005; Shah HR et al. 2006; Shah SR et al. 2010), gastric (Hata et al. 2016; Rich et al. 2004; Saif 2005; Shah HR et al. 2006), and colorectal cancers (Camidge et al. 2005; Copur et al. 2001; Hata et al. 2016; Kolesar et al. 1999; Rich et al. 2004; Saif 2005; Shah HR et al. 2006; Shah SR et al. 2010) for several years. We hypothesised that the time necessary until the onset of changes in PD parameters attributable to 5-FU and WF DDIs in the clinics might not be observed in most *in vitro* studies because of their short study periods (Tayag et al. 2022). Our previous study showed that PT-INR and PT-INR/dose ratio were significantly increased after 2–3 weeks (Tayag et al. 2022) consistent with previous studies (Copur et al. 2001; Kolesar et al. 1999; Saif 2005; Shah HR et al. 2006). The significant increase in PT-INR/dose strongly suggested that 5-FU inhibited WF metabolism (Tayag et al. 2022). Continuing our investigation, we hypothesised that if 5-FU inhibited CYP3A4 and 2C9 activity, blood pressure (BP) would decrease in patients given antihypertensives metabolised by CYP3A4 or 2C9 due to increased blood concentrations of these drugs. The calcium channel blocker (CCB) amlodipine is a well-known substrate of CYP3A4 that has been shown to also have inhibitory activity against CYP3A4 (Wang et al. 2016). Most angiotensin receptor II blockers (ARBs) are primarily metabolised by CYP2C9 (Kamiyama et al. 2007). Therefore, we also investigated the effects of additional co-administration of antihypertensives also metabolised by CYP3A4 and CYP2C9 using BP as a PD parameter.

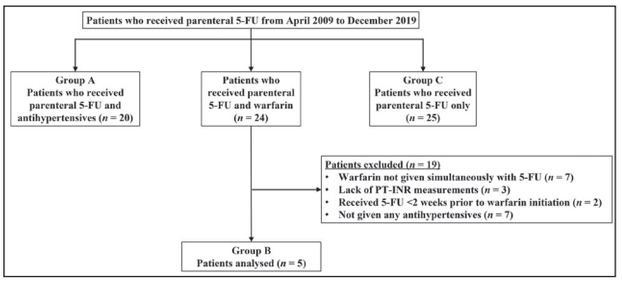

Fig. 1: Study design and flow chart for eligible patients 5-FU, 5-fluorouracil; PT-INR, prothrombin time international normalised ratio.

Table 1: Baseline patient characteristics

Characteristics	Group A		Group B		Group C	
	Total patients (n = 20)		Total patients (n = 5)		Total patients (n = 25)	
Male/Female (n)	15/5		2/3		18/7	
Age (years)	65 (60–71)		68 (59–76)		60 (55–69)	
Height (cm)	162 (154–165)		151 (147–157)		164 (157–168)	
Weight (kg)	60 (57–64)		56 (46–66)		57 (52–62)	
BSA (m^2)	1.64 (1.54–1.71)		1.53 (1.36–1.66)		1.59 (1.51–1.71)	
Chemotherapy cycle (n)						
14-day cycle	6		3		3	
21-day cycle	-		1		1	
28-day cycle	14		1		21	
Function test[a]	Pre-Chemo	During Chemo	Pre-Chemo	During Chemo	Pre-Chemo	During Chemo
AST (IU/L)	21.00 (16.00–29.00)	19.00 (15.00–30.00)	23.00 (20.00–45.50)	32.00 (14.50–45.00)	18.00 (15.00–24.00)	19.00 (16.00–25.00)
ALT (IU/L)	20.00 (10.00–30.00)	24.00 (14.00–32.00)	14.00 (10.50–32.00)	26.00 (19.00–51.50)	21.00 (12.00–28.00)	18.00 (12.00–34.00)
Crea (mg/dL)	0.80 (0.63–0.98)	0.82 (0.62–0.99)	0.84 (0.62–0.87)	0.78 (0.63–0.91)	0.66 (0.58–0.84)	0.66 (0.62–0.81)
eGFR (mL/min/1.73m^2)	74.70 (63.60–88.90)	73.10 (57.00–89.90)	68.30 (49.00–92.15)	71.80 (54.40–76.55)	78.60 (69.20–102.9)	80.60 (70.70–99.30)
5-FU (mg/day)						
median	1335 (1216–3277)		3500 (875–3550)		1250 (1152–1363)	
Warfarin daily dose (mg/day) (Pre-Chemo)						
median	N/A		1.50 (1.13–1.75)		N/A	
5-FU Indication (n)						
Colorectal cancer	5		3		3	
Head and neck cancer	14		1		19	
Oesophageal cancer	-		1		2	
Breast cancer	1		-		1	
Chemotherapy regimens (n)[b]						
NDP + 5-FU	14		1		19	
NDP + 5-FU + Radiation therapy	-		1		-	
FOLFIRI + Pmab	-		1		-	
FOLFIRI + BV (5)	2		2		-	
FOLFIRI + BV (10)	-		1		-	
FOLFIRI + Cmab (biweekly)	1		-		-	
FOLFIRI + RAM	-		-		1	
mFOLFOX-6	1		-			
mFOLFOX-6 pump	2		-		2	
mFOLFOX-6 + Pmab	1		-		-	
5-FU + CDDP	-		-		1	
CMF	-		-		1	
FAN	-		-		1	
Warfarin Indication (n)						
Atrial fibrillation	N/A		2		N/A	
Deep vein thrombosis	N/A		1		N/A	
Superior vena cava thrombosis	N/A		1		N/A	
Microthrombus/Catheter patency (prophylaxis)	N/A		1		N/A	

Data are n (number of patients) and median (interquartile range). Group A, 5-FU+Antihypertensives; Group B, 5-FU+WF+Antihypertensives; Group C, 5-FU Only; BSA, body surface area; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Crea, creatinine; eGFR, estimated glomerular filtration rate; NDP, nedaplatin; FOLFIRI, leucovorin calcium (folinic acid), fluorouracil, and irinotecan hydrochloride; Pmab, panitumumab; BV, bevacizumab; Cmab, cetuximab; RAM, ramucirumab; FOLFOX-6, leucovorin calcium (folinic acid), fluorouracil, and oxaliplatin; CDDP, cisplatin; CMF, cyclophosphamide, methotrexate, fluorouracil; FAN, fluorouracil, adriamycin, and nedaplatin.

[a] Changes within groups pre-chemotherapy and during chemotherapy were not statistically significant (paired t-test). There were no significant differences in pre-chemotherapy and during chemotherapy values between the three groups (Kruskal-Wallis with Dunn's multiple comparisons test).

[b] Some patients received multiple regimens.

2. Investigations and results

2.1. Patient characteristics

Figure 1 shows the study design and flow chart for identifying patients eligible for analysis. We identified 20 patients who received parenteral 5-FU chemotherapy in combination with antihypertensives metabolised by CYP3A4 or 2C9 (Group A) from April 2009 to December 2019 at the University of the Ryukyus Hospital. The antihypertensives prescribed were a) amlodipine (n = 11), nifedipine (n = 1), and amlodipine + nifedipine (n = 1); b) candesartan (n = 3) and valsartan (n = 1); and c) amlodipine + candesartan (n = 1), amlodipine + losartan (n = 1), and nifedipine + valsartan (n = 1). We also identified 24 patients who received parenteral 5-FU chemotherapy in combination with WF. A total of 19 patients did not meet the inclusion criteria. 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. Seven patients were excluded because they

Table 2: Antihypertensives and daily dosage

Characteristics	Group A Total patients (n = 20)	Group B Total patients (n = 5)
CYP3A4 Substrates	n = 13	n = 2
+ CCB	amlodipine (11) nifedipine (1) amlodipine + nifedipine (1)	amlodipine (2)
CYP2C9 Substrates	n = 4	-
+ ARB	candesartan (3) valsartan (1)	-
CYP3A4 + CYP2C9 Substrates	n = 3	n = 3
+ amlodipine + ARB	candesartan (1) losartan (1)	candesartan (1) valsartan (1) telmisartan (1)
+ nifedipine + ARB	valsartan (1)	-
Amlodipine daily dose (mg/day)	n = 14	n = 5
median	5.0 (4.4–6.3)	5.0 (5.0)
Nifedipine daily dose (mg/day)	n = 3	-
median	40.0 (20.0–40.0)	-
Candesartan daily dose (mg/day)	n = 4	n = 1
median	4.0 (4.0–7.0)	8
Valsartan daily dose (mg/day)	n = 2	n = 1
median	60.0 (40.0–80.0)	80
Losartan daily dose (mg/day) (n = 1)	100	-
Telmisartan daily dose (mg/day) (n = 1)	-	40

Data are n (number of patients) and median (interquartile range). CCB, calcium channel blocker; ARB, angiotensin II receptor blocker.

did not receive any antihypertensives. Five patients were eligible for analysis in the comparator group (Group B). Patients received 5-FU in combination with WF and antihypertensives, specifically, a) amlodipine (n = 2) and b) amlodipine + telmisartan (n = 1), amlodipine + candesartan (n = 1) and amlodipine + valsartan (n = 1). For the control group (Group C), we identified 25 patients who received 5-FU only. All patients did not receive any other established CYP3A4 or 2C9 inhibitors. The dosage of CCB or ARB received by each patient, where applicable, was maintained constantly throughout the study period.

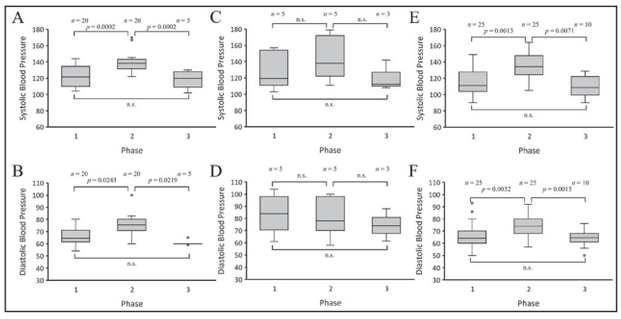

Fig. 2: Changes in SBP and DBP (peak levels during chemotherapy)
Phase 1, baseline pre-chemotherapy; Phase 2, peak during chemotherapy; Phase 3, at least 1-month post-chemotherapy; n.s., not statistically significant. Changes compared using one-way ANOVA with Tukey-Kramer test. (A-B) Group A, 5-FU+Antihypertensives. (C-D) Group B, 5-FU+WF+Antihypertensives. (E-F) Group C, 5-FU Only. *DBP values were based on the corresponding SBP measurements.

Tables 1 and 2 summarise the baseline patient characteristics and daily dosages. There were no significant changes in hepatic and renal function tests from baseline values pre-chemotherapy to those during 5-FU administration (Table 1). There were no bleeding complications, adverse events, or indications for rescue therapies for all patients.

2.2. Changes in blood pressure

The dose of antihypertensives was maintained throughout the analysed time periods. Regarding the peak BP during chemotherapy, in Group A, the median values of SBP and DBP significantly increased by 11% and 17% of the baseline values, respectively (Fig. 2). In Group B, SBP increased by 15% of the baseline value at peak levels during co-administration with WF and 5-FU. DBP decreased by 7% of the baseline value. Neither change was statistically significant. In Group C, SBP and DBP significantly increased by 19% and 16% of the baseline values, respectively.
As for the lowest BP levels during chemotherapy, in Group A, the median value of SBP significantly decreased by 19%, and DBP decreased by 8% of the baseline value, but it was not significant (Fig. 3).

Table 3: Changes in pre-chemotherapy blood pressure to during chemotherapy blood pressure levels (+antihypertensives)

Parameter	Group A Total patients (n = 20)			Group B Total patients (n = 5)		
Changes from pre-chemotherapy blood pressure to peak blood pressure during chemotherapy						
	Pre-Chemo	During Chemo	P value	Pre-Chemo	During Chemo	P value
CYP3A4 substrates (CCB) (n = 13)				CYP3A4 substrates (Amlodipine only) (n = 2)		
SBP (mmHg)						
median	123 (107–135)	134 (127–142)	0.0015	134 (111–157)	147 (122–172)	n.s.
DBP (mmHg)						
median	64 (61–71)	76 (70–80)	0.0002	94 (84–104)	89 (78–100)	n.s.
CYP2C9 substrates (ARB) (n = 4)				CYP2C9 substrates (ARB)		
SBP (mmHg)						
median	119 (112–138)	142 (130–145)	n.s.	N/A		
DBP (mmHg)						
median	72 (63–79)	77 (67–95)	n.s.	N/A		
CYP3A4 (CCB) + CYP2C9 (ARB) substrates (n = 3)				CYP3A4 (Amlodipine) + CYP2C9 (ARB) substrates (n = 3)		
SBP (mmHg)						
median	126 (122–142)	141 (140–144)	n.s.	120 (103–154)	138 (111–179)	n.s.
DBP (mmHg)						
median	67 (60–70)	75 (60–83)	n.s.	71 (61–98)	70 (58–98)	n.s.
Changes from pre-chemotherapy blood pressure to lowest blood pressure during chemotherapy						
CYP3A4 substrates (CCB) (n = 13)				CYP3A4 substrates (Amlodipine only) (n = 2)		

SBP (mmHg)						
median	123 (107–135)	100 (89–109)	<0.0001	134 (111–157)	115 (99–130)	n.s.
DBP (mmHg)						
median	64 (61–71)	57 (53–70)	n.s.	94 (84–104)	70 (62–77)	n.s.
CYP2C9 substrates (ARB) (n = 4)				CYP2C9 substrates (ARB)		
SBP (mmHg)						
median	119 (112–138)	95 (87–108)	n.s.	N/A		
DBP (mmHg)						
median	72 (63–79)	60 (60–75)	n.s.	N/A		
CYP3A4 (CCB) + CYP2C9 (ARB) substrates (n = 3)				CYP3A4 (Amlodipine) + CYP2C9 (ARB) substrates (n = 3)		
SBP (mmHg)						
median	126 (122–142)	110 (100–110)	n.s.	120 (103–154)	99 (98–158)	n.s.
DBP (mmHg)						
median	67 (60–70)	60 (48–70)	n.s.	71 (61–98)	55 (64–97)	n.s.

Data are n (number of patients), median (interquartile range), and P value. SBP, systolic blood pressure; DBP, diastolic blood pressure; CCB, calcium channel blocker; ARB, angiotensin receptor II blockers; n.s., not significant. Changes within groups pre-chemotherapy and during chemotherapy were compared using paired t-test.

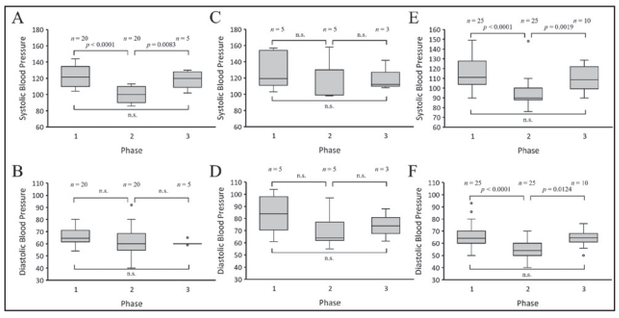

Fig. 3: Changes in SBP and DBP (lowest levels during chemotherapy)
Phase 1, baseline pre-chemotherapy; Phase 2, lowest during chemotherapy; Phase 3, at least 1-month post-chemotherapy; n.s., not statistically significant. Changes compared using one-way ANOVA with Tukey-Kramer test. (A-B) Group A, 5-FU+Antihypertensives. (C-D) Group B, 5-FU+WF+Antihypertensives. (E-F) Group C, 5-FU Only. *DBP values were based on the corresponding SBP measurements.

In Group B, SBP and DBP decreased by 18% and 24% of the baseline values, respectively, at the lowest levels during co-administration with WF and 5-FU. In Group C, SBP and DBP significantly decreased by 20% and 16% of the baseline values, respectively.

Additional analyses according to type of antihypertensive for BP control was performed (Table 3). In patients who received CCBs in Group A, regarding the peak BP levels during chemotherapy, both SBP and DBP significantly increased by 9% and 19% of the baseline values, respectively. In contrast, patients who received CCB (amlodipine) in Group B showed an increase in SBP by 10% and a decrease in DBP by 5% of the baseline value, respectively, but neither change was significant. As for the lowest BP levels during chemotherapy, in patients who received CCBs in Group A, SBP significantly decreased by 19%, and DBP decreased 11% of the baseline values, respectively. Patients who received CCB (amlodipine) in Group B showed a decrease in SBP by 14% and a decrease in DBP by 29% of the baseline value, respectively, but neither change was significant.

Analyses of patients who received ARBs and multiple antihypertensives were also performed, but the results were not statistically significant due to the small sample sizes. After completion of chemotherapy, while administering WF and antihypertensives, the SBP and DBP returned to baseline values (Fig. 2, Supporting Information, Fig. S1).

2.3. Changes in WF daily dose, PT-INR, and PT-INR/dose

For Group B, the changes in baseline WF daily dose before chemotherapy were compared with the minimum dose administered within a chemotherapy cycle. The median daily dose of WF decreased by 33% of the pre-chemotherapy dose, but the change was not statistically significant (Table 4). PT-INR significantly increased by 91% of the baseline value at peak levels during co-administration of WF with 5-FU. PT-INR/dose increased by 45% of the baseline value, but it was not statistically significant. There were no statistically significant changes in PT-INR in Groups A and C. The median 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 5. The median time to peak PT-INR and peak PT-INR/dose was at least 2.5 weeks from co-administration with 5-FU. The elevated PT-INR and PT-INR/dose returned to baseline values after completion of chemotherapy (Supporting Information, Fig. S3). Antihypertensives were administered continuously.

3. Discussion

We hypothesised that if 5-FU inhibited CYP3A4 and 2C9 activity, BP would decrease due to increasing blood concentrations of antihypertensives. In this study, we investigated the effect of 5-FU DDIs on antihypertensive drugs also metabolised by CYP2C9 and 3A4. In Group A and C, the peak SBP and DBP values significantly increased compared to the baseline values and the values after completion of 5-FU administration (Fig. 2, Supporting Information, Fig. S1). The lowest SBP and DBP values also decreased compared to the baseline values and the values after completion of 5-FU administration for Groups A and C, but the decrease in DBP was not significant in Group A (Fig. 3, Supporting Information, Fig. S2). These observations suggest possible DDIs between 5-FU and antihypertensive drugs, although there were no significant changes in Group B. Although the PT-INR and PT-INR/dose also increased following co-administration of WF and antihypertensives with 5-FU, only the elevation in PT-INR was significant. These observations in Group B can possibly be due to the smaller sample size of the current study. Consistent with our previous findings (Tayag et al. 2022), these changes were reversible after completion of 5-FU administration, and the lack of significant changes in PT-INR in Groups A and C confirms the DDI between 5-FU and WF.

To the best of our knowledge, this is the first study to use BP changes as a PD marker to evaluate the DDIs between 5-FU and antihypertensives metabolised by CYP3A4 and CYP2C9. Although CCBs are known as substrates and inhibitors of CYP3A4 (Štěpánková et al. 2016; Wang et al. 2016), there are yet to be any reports on the effects on BP during co-administration of 5-FU with CCBs. The cohort study of Wang et al. (2016) analysed the increased risk of adverse events in the co-administration of CYP3A4-metabolised statins and CYP3A4-inhibiting CCBs; however, the study did not investigate changes in BP directly.

Table 4: Changes in warfarin parameters after 5-FU co-administration

Parameter	Group A			Group B			Group C		
	Total patients (n = 6)			Total patients (n = 5)			Total patients (n = 7)		
	Pre-Chemo	During Chemo	P value	Pre-Chemo	During Chemo	P value	Pre-Chemo	During Chemo	P value
Warfarin daily dose (mg/day)									
median		N/A		1.50 (1.13–1.75)	1.00 (1.00–1.75)	n.s.		N/A	
PT-INR[a]									
median	1.14 (1.07–1.25)	1.23 (1.16–1.28)	n.s.	1.28 (1.04–1.52)	2.45 (1.45–2.81)	0.0245	1.03 (0.95–1.11)	1.09 (1.09–1.13)	n.s.
PT-INR/dose[b]									
median		N/A		0.85 (0.67–1.07)	1.23 (1.03–2.24)	n.s.		N/A	

Data are n (number of patients), median (interquartile range), and P value. PT-INR, prothrombin time international normalised ratio; n.s., not significant
[a] Changes pre-chemotherapy and during chemotherapy were compared using paired t-test.
[b] Changes pre-chemotherapy and during or after chemotherapy were compared using paired t-test.

Table 5: Time course of events after 5-FU co-administration

Event	Group A	Group B	Group C
	Total patients (n = 20)	Total patients (n = 5)	Total patients (n = 25)
Days to peak BP			
median	14.0 (5.5–22.5)	15.0 (6.0–61.5)	15.0 (6.0–48.0)
Days to lowest BP			
median	20.5 (7.5–27.0)	26.0 (12.0–52.0)	17.0 (8.0–31.5)
Days to peak PT-INR			
median	41.5 (17.3–47.8) [a]	18.0 (12.5–52.5)	41.0 (17.0–65.0) [b]
Days to peak PT-INR/dose			
median	N/A	18.0 (16.0–60.5)	N/A
Days of 5-FU & WF co-administration/chemo cycle			
median	N/A	1.0 (1.0–7.5)	N/A
Days after end of co-administration to first WF dose adjustment			
median	N/A	9.0 (0.0–10.5)	N/A

Data are n (number of patients) and median (interquartile range). PT-INR, prothrombin time international normalised ratio; BP, blood pressure. Peak BP refers to the highest SBP and its corresponding DBP. There were no significant differences in values between the three groups (Kruskal-Wallis with Dunn's multiple comparisons test).
[a] n = 6
[b] n = 7

Additional inhibition of CYP3A4 and 2C9 by amlodipine would have produced more pronounced hypotension. Using human liver microsomes, Krasulová et al. (2017) demonstrated that although amlodipine is itself a substrate of CYP3A4, amlodipine exhibited both competitive and time-dependent inhibition of CYP3A4. Amlodipine was also demonstrated to be a weak to moderate inhibitor of CYP2C9 and CYP2C19 activity (Krasulová et al. 2017). However, our results conflictingly showed a significant increase in SBP. Hypertension is one of the vascular complications associated with several chemotherapeutic regimens (Campia et al. 2019; Cameron et al. 2016; Daher and Yeh 2008). The significant increase in SBP can be attributed to chemotherapy-induced hypertension by 5-FU or other drugs in the chemotherapeutic regimens. This can be confirmed by the significant increases in SBP and DBP in Groups A and C. SBP also increased in Group B, but DBP decreased. Although some patients received additional antihypertensives, such as an angiotensin-converting-enzyme inhibitor (enalapril) or a diuretic (furosemide), patients who did not receive these additional interventions also had a decrease in DBP. Instead, this decrease in DBP may be due to amlodipine's efficacy in lowering DBP compared to other antihypertensives (Eguchi et al. 2004; Lenz et al. 2001; Mehlum et al. 2020; Radauceanu et al. 2004; Shi et al. 2017; Solanki et al. 2021), and the additional co-administration of WF in Group B may suggest additional DDI between WF and amlodipine. Moreover, when comparing the patients who received CCBs, although SBP also increased for patients in Group B (Table 3), the increase was not statistically significant, unlike in Group A. It is possible that the antihypertensive effect of amlodipine may have been enhanced. Another explanation is that the antihypertensive drugs in the study are less effective in decreasing SBP and are more effective in decreasing the more easily reversible DBP (Mancia et al. 2002). However, when comparing the lowest BP levels during chemotherapy, there was a decrease in SBP and DBP in all groups from the baseline values. In particular, there was a significant decrease in SBP among patients who received CCBs in Group A (Table 3). The median time to peak and lowest BP was at least 2 weeks and 3 weeks, respectively, for all groups (Table 5), suggesting that a BP lowering effect was observed following the offset of the initial chemotherapy-induced hypertension. The return of SBP and DBP to baseline values at least 1 month after completion of 5-FU administration in all groups might reflect the time until the offset of DDI effects (Fig. 2 and 3, Supporting Information, Fig. S1 and S2).

It is possible that rather than the actual time frame of DDI, the BP recordings reflect the monitoring schedule. BP was routinely measured prior to 5-FU administration according to the protocol of each chemotherapy regimen. However, particularly for out-patients, BP readings during chemotherapy were taken only at intervals of 14, 21, or 28 days, according to the chemotherapy cycle. BP measurements between day 1 and the last day of the chemotherapy cycle were not reported for these cases. BP measurements after completion of chemotherapy were measured according to the follow-up schedule.

Recent literature suggests that 5-FU and CYP DDIs might involve molecular mechanisms in which 5-FU affects DNA synthesis and interferes with normal RNA function of CYPs (Curtin et al. 1991; Jin et al. 2016; Longley et al. 2003; Marin-Vicente et al. 2013; Mojardín et al. 2013; Pellino and Danenberg 1985; Peters et al. 2000). In our previous investigation of 5-FU inhibition of PK-related gene expression, there was a significant reduction in CYP3A4

mRNA levels in HepaRG cells exposed to 10 µg/mL 5-FU for 96 h (Shiohira et al. 2021). A significant reduction in CYP3A4 mRNA levels would lead to a downregulation of CYP3A4 enzymes and, consequently, an increase in amlodipine blood concentration. This may lead to the possibility of enhancing the antihypertensive effect of amlodipine which may account for the changes in BP observed in Group B. Although there was no significant change in CYP2C9 mRNA levels, there was also a significant reduction of mRNA levels of nuclear receptor subfamily 1 group I member 2 (NR1I2), a regulator of CYPs (Shiohira et al. 2021). With prolonged exposure, as seen in the clinics, we expect to see a significant reduction in CYP2C9 mRNA levels as well.

The elevations in PT-INR and PT-INR/dose from the pre-chemotherapy values to those during co-administration with 5-FU were similar to those in our previous retrospective study (Tayag et al. 2022). In our previous study, the mean WF daily doses before and during chemotherapy were 2.61 mg/day and 1.75 mg/day, respectively (Tayag et al. 2022). The current study showed a more significant increase in PT-INR despite the lower mean WF daily doses before chemotherapy and during chemotherapy, 1.45 mg/day and 1.30 mg/day, respectively. These PT-INR and PT-INR/dose elevations were not only consistent with our previous findings, but these elevations showed a further upward trend compared to the elevations in the previous study, which may be attributed to the additional co-administration of antihypertensives.

In clinical studies, the effects of 5-FU and WF DDIs appeared after at least a few weeks (Copur et al. 2001; Kolesar et al. 1999; Saif 2005; Shah HR et al. 2006; Tayag et al. 2022). In our previous study, the median times to peak PT-INR and peak PT-INR/dose were at least 2 and 3.5 weeks, respectively (Tayag et al. 2022). In our current study, the median times to both peak PT-INR and peak PT-INR/dose were at least 2.5 weeks (Table 5). This was consistent with previous studies. Human hepatic CYP2C9 and 3A4 have turnover half-lives of approximately 104 h and 44–140 h, respectively (Yang et al. 2008). Thus, the complete turnover of normally functioning CYP enzymes would occur at least after 2–3 weeks, and the median time to peak PT-INR of 2.5 weeks coincided with this estimate. Owing to this turnover, 5-FU DDI effects in clinical settings may require a long time before they can be observed. The time until complete turnover of CYP enzymes can also explain why BP parameters returned to baseline values at least 1 month after completion of 5-FU administration (Fig. 2, Supporting Information, Fig. S1) as normally functioning CYP enzymes should have recovered to their original amount. Further studies on long-term 5-FU exposure, which have not yet been done *in vitro*, are needed to confirm the data in this clinical study.

The limitations of this study include the small sample size, low doses of WF (1 mg/day) with no comparison with higher doses, and discontinuity in WF therapy. Further studies with larger sample sizes are needed to determine the effects of additional co-administration of other CYP3A4 or CYP2C9 substrates.

This study shows that co-administration of parenteral 5-FU, WF, and antihypertensives led to a decrease in the peak DBP, the lowest SBP, and the lowest DBP levels during chemotherapy compared to the baseline values. The findings suggest possible DDIs between 5-FU and antihypertensives metabolised by CYP3A4. Therefore, knowledge of the DDIs of these classes of antihypertensives during 5-FU chemotherapy will be useful to clinicians and pharmacists.

4. Experimental

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 number 1102).

As in our previous study (Tayag et al. 2022), 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. The following patient information were reviewed and analysed: sex, age, height, weight, body surface area, length of chemotherapy cycle, indication for 5-FU, chemotherapy regimen, dose of 5-FU, co-administration and dose of antihypertensives also metabolised by CYP3A4 or CYP2C9, systolic blood pressure (SBP), diastolic blood pressure (DBP), PT-INR, and laboratory indicators of hepatic and renal function. Chemotherapy was administered in cycles of 14, 21, or 28 days. Some patients received multiple cycles of chemotherapy. The inclusion criteria for Group A were as follows: 1) We identified patients who received 5-FU co-administered with antihypertensives, specifically, a) CCBs, b) ARBs, or c) a combination of CCB + ARB. 2) Only patients who did not receive any known CYP3A4 or 2C9 inhibitors other than amlodipine were analysed. Although candesartan and telmisartan showed moderate inhibitory activity against CYP2C9 *in vitro*, it was not expected to produce any effects at clinical dosages (Kamiyama et al. 2007). We also identified all the patients who received 5-FU chemotherapy in combination with antihypertensives and WF for the comparator group (Group B). Additional patient information reviewed and analysed included indication for WF and dose of WF. The PT-INR/dose ratio was used to evaluate the WF titre (Tayag et al. 2022). As an additional inclusion criterion for Group B, to account for a washout period of 14 days (Ikenishi et al. 2016), only patients who had no 5-FU exposure for at least 2 weeks prior to WF initiation were included. Patients who received 5-FU alone were identified and analysed as the control group (Group C).

Data are expressed as median (interquartile range) unless otherwise stated. For the SBP and DBP, all DBP values were based on the corresponding SBP measurements. For the WF daily dosage, the baseline values before chemotherapy refer to the WF dose given on or closest to Day 0 of chemotherapy, and the values during chemotherapy refer to the minimum dosage of WF given within a chemotherapy cycle. For the PT-INR, the baseline values refer to the PT-INR reported on or closest to Day 0 of chemotherapy, and the peak values refer to the highest PT-INR reported within a chemotherapy cycle. For the PT-INR/dose, values were calculated based on the available data. Baseline values refer to the PT-INR/dose on or closest to Day 0 of chemotherapy, and peak values refer to the highest PT-INR/dose either within a chemotherapy cycle or after the last day of the cycle. Changes following co-administration of 5-FU in hepatic and renal function indicators, SBP, DBP, PT-INR, WF daily dosage, and PT-INR/dose were analysed using paired t-test. Differences in values between Group A, B, and C and the differences between BP values pre-chemotherapy, during chemotherapy, and 1 month after chemotherapy within groups were analysed using Kruskal-Wallis test with Dunn's multiple comparisons test or one-way analysis of variance (ANOVA) with Tukey-Kramer test as appropriate. All P values were set at <0.05 for statistical significance. Statistical analyses were performed using GraphPad Prism version 8.0.2 for Windows (GraphPad Software, San Diego, CA, USA).

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Conflict of Interest: The authors declare that they have no competing interests.

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Pharmazie

Effects of 5-Fluorouracil Co-Administration on Blood Pressure in Patients Maintained on Antihypertensives: A Retrospective Case Series

Supporting Information

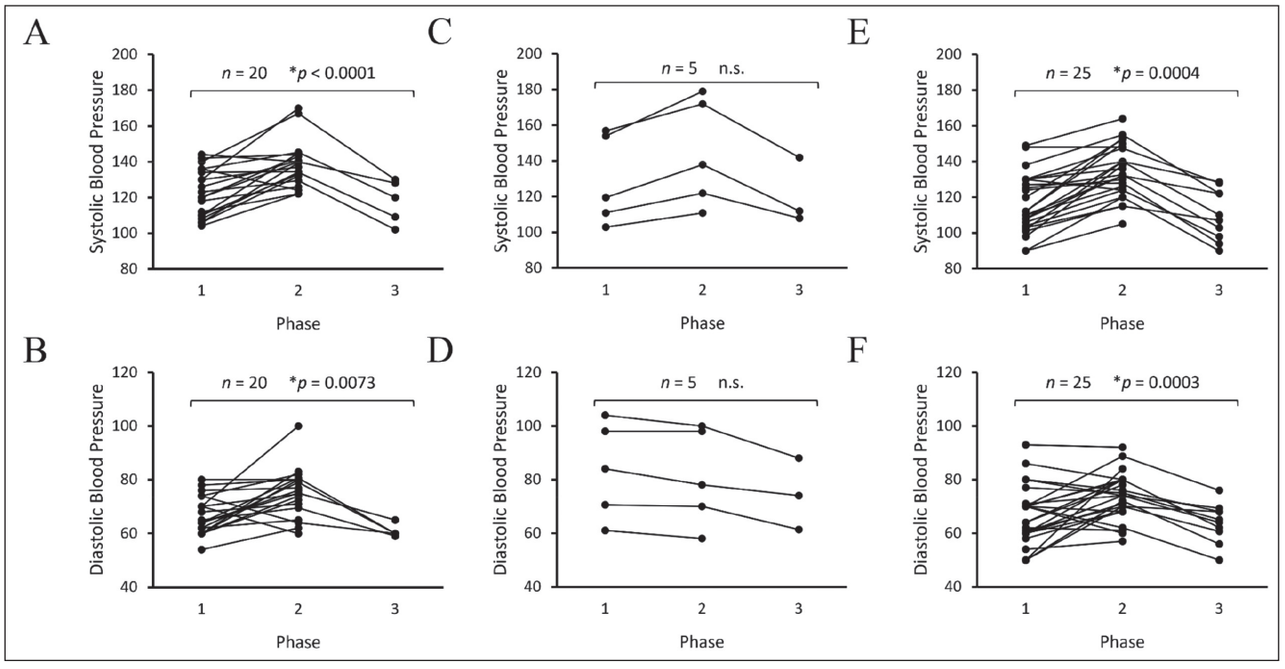

Fig. S1: Changes in SBP and DBP (peak levels during chemotherapy). Patients shown individually.
Phase 1, baseline pre-chemotherapy; Phase 2, peak during chemotherapy; Phase 3, at least 1-month post-chemotherapy; n.s., not statistically significant. Changes compared using one-way ANOVA with Tukey-Kramer test. (A-B) Group A, 5-FU+Antihypertensives. (C-D) Group B, 5-FU+WF+Antihypertensives. (E-F) Group C, 5-FU Only. *DBP values were based on the corresponding SBP measurements.

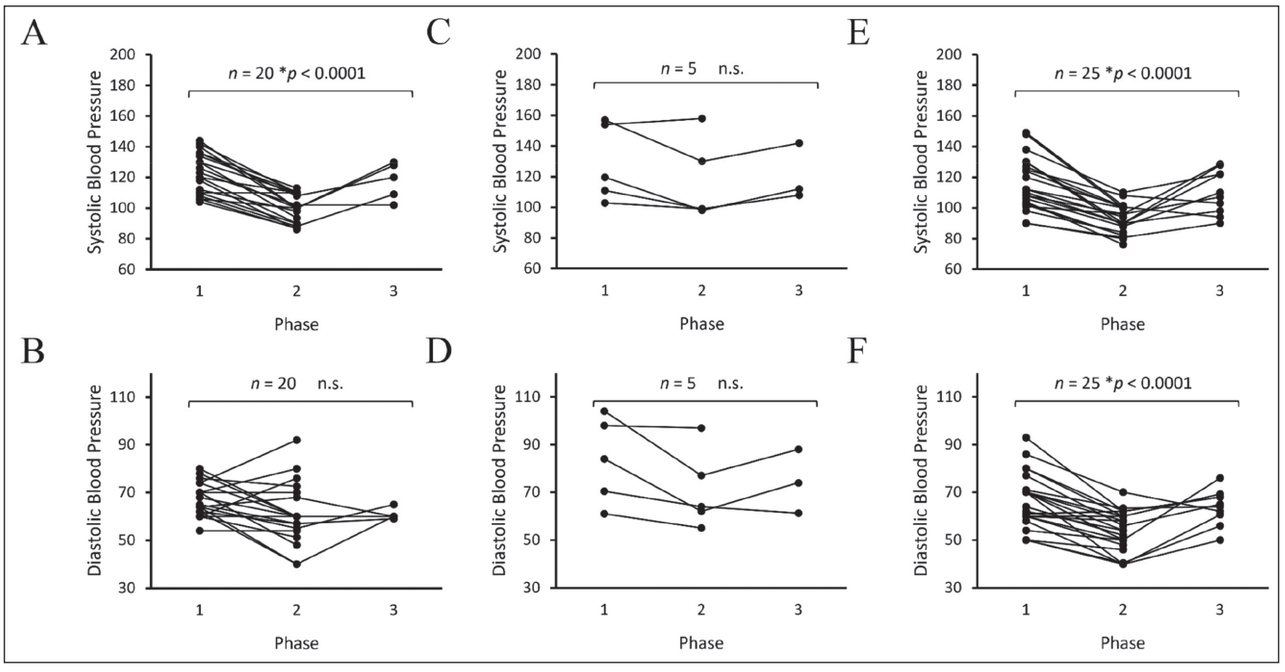

Fig. S2: Changes in SBP and DBP (lowest levels during chemotherapy). Patients shown individually.
Phase 1, baseline pre-chemotherapy; Phase 2, lowest during chemotherapy; Phase 3, at least 1-month post-chemotherapy; n.s., not statistically significant. Changes compared using one-way ANOVA with Tukey-Kramer test. (A-B) Group A, 5-FU+Antihypertensives. (C-D) Group B, 5-FU+WF+Antihypertensives. (E-F) Group C, 5-FU Only. *DBP values were based on the corresponding SBP measurements.

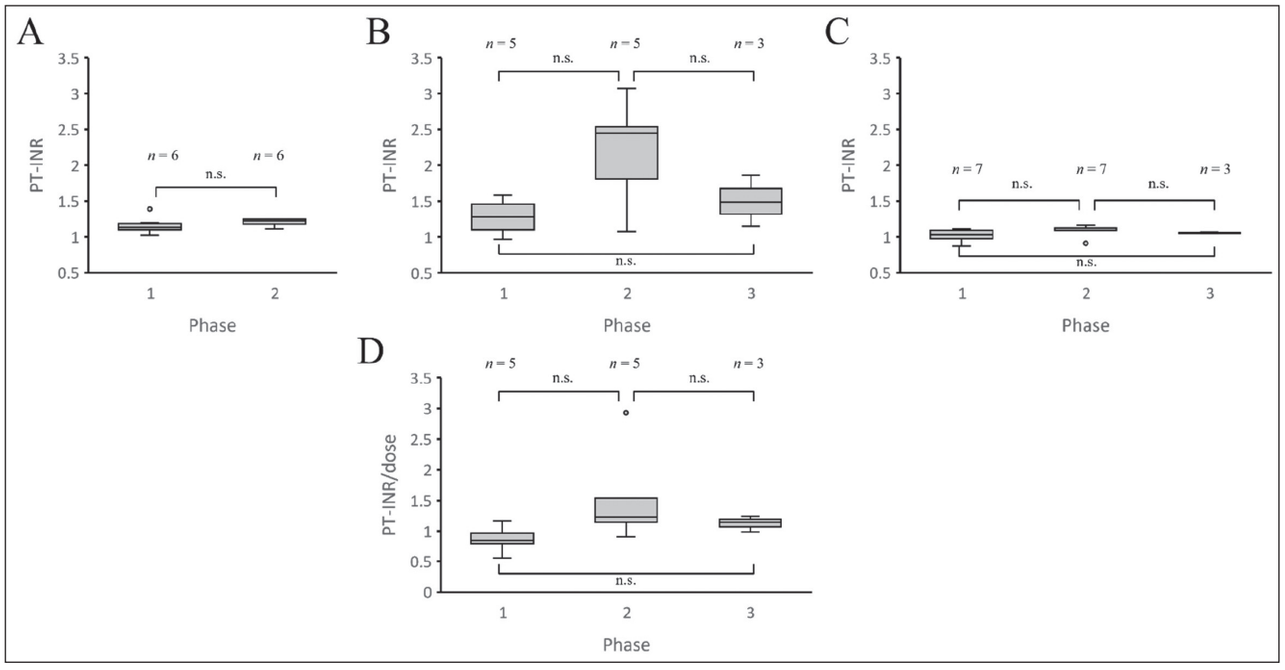

Fig. S3: Changes in PT-INR and PT-INR/dose.
Phase 1, baseline pre-chemotherapy; Phase 2, peak during chemotherapy; Phase 3, at least 1-month post-chemotherapy; n.s., not statistically significant. Changes in PT-INR: (A) Group A, 5-FU+Antihypertensives (paired t-test); (B) Group B, 5-FU+WF+Antihypertensives (Kruskal-Wallis with Dunn's multiple comparisons test); (C) Group C, 5-FU Only (Kruskal-Wallis with Dunn's multiple comparisons test). (D) Changes in PT-INR/dose (Kruskal-Wallis with Dunn's multiple comparisons test); peak PT-INR/dose does not necessarily correspond to peak PT-INR.