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ORIGINAL ARTICLE

Effects of bevacizumab on plasma concentration of irinotecan and its metabolites in advanced colorectal cancer patients receiving FOLFIRI with bevacizumab as second-line chemotherapy

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Abstract
Purpose Bevacizumab (BV) prolongs the survival of colorectal cancer patients when combined with irinotecan (CPT-11)-based regimens. In the AVF2107g study, the area under the curve (AUC) ratio for bolus CPT-11/5-fluorouracil (5-FU)/leucovorin (LV) (IFL) with the BV arm to bolus IFL with placebo indicated that SN-38 concentrations may have been increased in subjects receiving BV. However, the mechanism underlying such increase remains unclear, and the difference might be caused by an imbalance between the two arms and a possible inter-subject variability of CPT-11 metabolism. Within-subject comparisons were used to evaluate the effect of BV on advanced colorectal cancer patients when administered with the FOLFIRI regimen as second-line chemotherapy.
Methods Ten advanced colorectal cancer patients received the FOLFIRI regimen every 2 weeks. At cycle 1, BV was administered following FOLFIRI administration to allow baseline pharmacokinetic (PK) analysis of CPT-11 and its metabolites. From cycle 2, BV was administered just before FOLFIRI administration. Plasma samples were collected under the same condition (at cycle 3).
Results There were no significant differences in the C_{max} and $AUC_{0-\infty}$ of CPT-11, SN-38, and SN-38G between cycle 1 (without BV) and cycle 3 (with BV). PK parameters of CPT-11, SN-38, and SN-38G were not significantly affected by BV. There were no significant differences in the changes in the AUC ratio of CPT-11 to SN-38 between cycles 1 and 3, as well as in the ratio of SN-38 to SN-38G.
Conclusion BV does not affect the plasma concentration of CPT-11 and its metabolites on FOLFIRI regimen.

Keywords Bevacizumab (BV) · Irinotecan · Pharmacokinetics · Colorectal cancer

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Introduction

Bevacizumab (BV) is a humanized monoclonal antibody against vascular endothelial growth factor, an important regulator of physiologic and pathologic angiogenesis [1]. A large, randomized, controlled Phase III clinical trial (AVF2107g) has demonstrated that BV addition to standard chemotherapy with the bolus irinotecan (CPT-11)/5-fluorouracil (5-FU)/leucovorin (LV) (IFL) regimen improves survival of patients with previously untreated metastatic colorectal cancer [2]. Subsequently, CPT-11/bolus 5-FU/continuous 5-FU/LV (FOLFIRI) + BV conferred a significant survival benefit compared with IFL + BV in the BICC-C study [3]. Thus, CPT-11 with BV demonstrated significant survival benefits in patients with colorectal cancer. CPT-11 has a complex metabolism requiring activation into SN-38 by carboxylesterase [4, 5] and glucuroconjugation for catabolism [6]. As shown in the AVF2107g study, SN-38 concentrations were on average 33% higher in patients receiving bolus IFL in combination with BV compared with bolus IFL alone [7]. However, the underlying mechanism of such increase remains unclear, and the difference might be caused by an imbalance between the two arms and a possible inter-subject variability of CPT-11 metabolism. Thus, we investigated the potential pharmacokinetic (PK) interaction between CPT-11 and BV in advanced

colorectal cancer patients when administered with the FOLFIRI + BV regimen as second-line chemotherapy.

Methods

Inclusion and exclusion criteria

Patients meeting the following inclusion criteria were eligible: histologically proved colorectal cancer (e.g., adenocarcinoma, mucinous carcinoma, and signet-ring cell carcinoma); failure of first-line treatment containing 5-FU-based chemotherapy (almost an adjuvant setting and recurrence were found in the chemotherapy period or after the end of chemotherapy within 24 weeks) or oxaliplatin-based chemotherapy (all FOLFOX regimens) without BV and CPT-11; Eastern Cooperative Oncology Group performance status of 0–2; age: 20–74-year-old; no previous exposure to BV or CPT-11; adequate bone marrow function (leukocyte count $\geq$3,000 and $\leq$12,000/μl, hemoglobin $\geq$8.0 g/dl, and platelet count $\geq 10 \times 10^4$/μl); serum creatinine level $\leq$1.5 mg/dl; total bilirubin level $\leq$1.5 mg/dl; AST and ALT $\leq$100 IU/l; qualitative urine protein $\leq$(1+); measurable disease according to response evaluation criteria for solid tumors (RECIST); and written informed consent.

Patients were excluded if they had the following: known central nervous system metastasis; other active double cancer; inadequately controlled hypertension, diarrhea, diabetes, or heart disease; severe peritoneal metastasis; interstitial pneumonia or pulmonary fibrosis; previous history of vascular thromboembolism or severe drug hypersensitivity; bleeding tendency; hepatic B or C virus infection; underwent any form of surgery within 4 weeks before study enrollment; pregnant or lactating.

Study design

Ten patients were treated with the FOLFIRI regimen preceded by BV every 2 weeks. At cycle 1, CPT-11 was administered before BV to allow baseline PK analysis of CPT-11 and its metabolites. At cycle 3, plasma samples were collected for PK analysis of CPT-11 when administered in combination with BV. The PK investigations were used intra-patients comparison.

Pretreatment and follow-up examination

Complete medical history evaluation, physical examination, laboratory tests (complete blood count, creatinine, serum electrolytes, calcium, uric acid, total protein, albumin level, hepatic, and coagulation tests) and urinalysis were performed to obtain baseline data and repeated biweekly.

Toxicity was evaluated biweekly and graded using the National Cancer Institute's Common Toxicity Criteria, version 3.0. Tumor responses were evaluated and measured as baseline data and reassessed every 4 cycles using RECIST.

Drug administration

The FOLFIRI regimen consisted of CPT-11 (180 mg/m^2 IV over 90 min), *l*-LV (200 mg/m^2 IV over 2 h), and 5-FU (400 mg/m^2 IV bolus), followed by 5-FU (2,400 mg/m^2 IV over a 46-h infusion), and repeated every 2 weeks. BV was administered as a 30-min intravenous infusion at a biweekly dose of 10 mg/m^2 before the FOLFIRI regimen (only in the cycle 1, BV was administered after the FOLFIRI regimen for PK analysis of the non-BV phase) (Fig. 1).

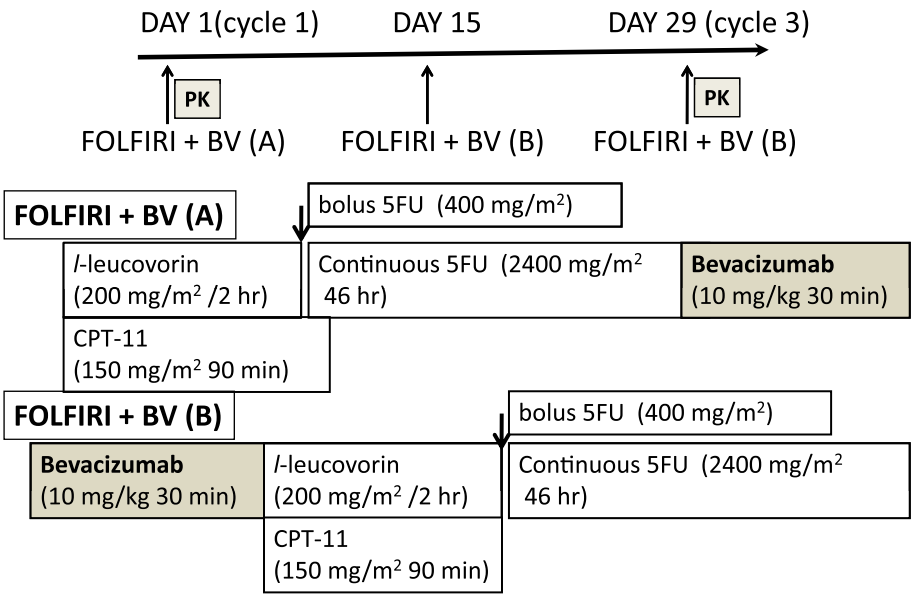

Fig. 1 At cycle 1, CPT-11 was administered before BV to allow baseline pharmacokinetic (PK) analysis of CPT-11 and its metabolites. At cycle 3, plasma samples were collected for PK analysis of CPT-11 when administered in combination with BV

Table 1 Patient characteristics

Age (years)	
Range	38–74
Median	60
Gender	
Male	9
Female	1
Previous chemotherapy	
5-FU-based regimen[a]	5
FOLFOX	5
Total cycles of treatment	
Range	7–19
Median	11

[a] As adjuvant chemotherapy

Pharmacokinetic analysis

Plasma samples were collected at cycles 1 and 3 before the start of chemotherapy, and 0, 1, 2, 4, 7, and 24 h after CPT-11 infusion. Whole blood (4.0 ml) samples were collected in heparinized tubes and centrifuged at 3,000 rpm for 10 min at 4°C. Then, 2.0 ml of plasma was transferred into tubes with 2.0 ml of phosphate buffer (0.1 M) and stored at −80°C before analysis. Thereafter, quantitative analysis of CPT-11 and its metabolites was performed using high-performance liquid chromatography [8]. The lower limit of quantification was 0.002 μg/ml for CPT-11 and its metabolites. Maximum plasma concentration (Cmax), area under the plasma/serum concentration time curve (AUC) and terminal half-life were determined. The AUC calculation is limited up to 24 h of to infinite (∞). Changes in the ratios of CPT-11 to SN-38 and SN-38 to SN-38G were estimated as $AUC_{SN\text{-}38}/AUC_{CPT\text{-}11}$ and $AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$, respectively.

Statistical analysis

Correlation between related species were all carried out using the paired *t* test (Microsoft Excel 2000 SP-3), and *P* values <0.05 with a two-tailed distribution were considered significant.

Results

Patient characteristics

Ten patients received the treatment regimens (Table 1), and all the patients completed the PK program and were assessable for drug safety and anti-tumor activity. A total of 120 cycles of treatment was administered (median number of cycles: 11 (range 7–19)).

Pharmacokinetic analysis

Analysis of the PK parameters showed no significant difference between the parameters of cycle 1 (non-BV phase) and cycle 3 (BV phase) (Table 2). This indicates that BV had no effect on the pharmacokinetics of CPT-11. The mean AUCs for CPT-11 were 12.2 ± 2.3 μg h/ml at cycle 1 and 12.8 ± 1.7 μg h/ml at cycle 3. The half-lives of CPT-11 were 6.0 ± 0.6 h at cycle 1 and 5.7 ± 0.6 h at cycle 3. Mean CPT-11 concentrations versus time profiles either alone or in combination with BV were nearly superimposed (Fig. 2).

The mean SN-38 PK parameters showed no significant differences between cycles 1 and 3 (Table 2). The mean AUCs for SN-38 were 0.40 ± 0.44 μg h/ml at cycle 1 and 0.22 ± 0.16 μg h/ml at cycle 3. Mean SN-38 concentrations versus time profiles either alone or in combination with BV were nearly superimposed (Fig. 3). In SN-38G, significant differences in the PK parameters were also not found between cycles 1 and 3 (Table 2), and mean SN-38G concentrations versus time profiles either alone or in

Table 2 Pharmacokinetic parameters

Analyte		C_{max} (mg/ml)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (mg h/ml)	$MRT_{0-\infty}$ (h)	Vd (L)	CL (L/h)
CPT-11	BV (−)	2.1 (0.3)	1.5 (0)	6.0 (0.6)	12.2 (2.3)	6.1 (0.6)	185 (43.3)	21.6 (5.6)
	BV (+)	2.1 (0.3)	1.5 (0)	5.7 (0.6)	12.8 (1.7)	6.1 (0.5)	164 (34.6)	19.7 (3.0)
SN-38	BV (−)	0.024 (0.013)	2.0 (0.7)	14.3 (16.6)	0.40 (0.44)	–	–	–
	BV (+)	0.022 (0.012)	2.8 (0.8)	8.3 (7.6)	0.22 (0.16)	–	–	–
SN-38G	BV (−)	0.14 (0.030)	2.4 (0.3)	12.9 (4.7)	1.98 (0.70)	–	–	–
	BV (+)	0.14 (0.030)	2.6 (0.6)	11.4 (3.5)	1.81 (0.26)	–	–	–

Values are expressed as mean (±SD). There are no significant differences in the C_{max} and $AUC_{0-\infty}$ of CPT-11, SN-38, and SN-38G between cycle 1 (BV-) and cycle 3 (BV+); paired *t* test

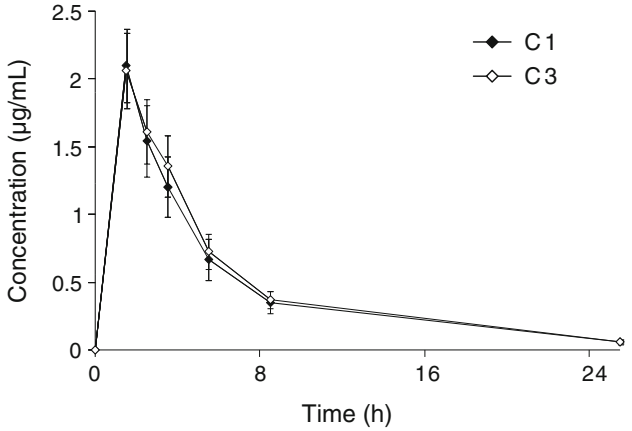

Fig. 2 Mean CPT-11 concentrations versus time profiles either alone or in combination with BV were superimposed

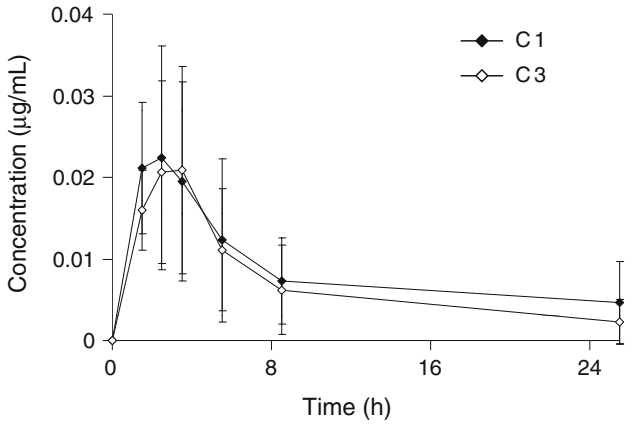

Fig. 3 Mean SN-38 concentrations versus time profiles either alone or in combination with BV were nearly superimposed

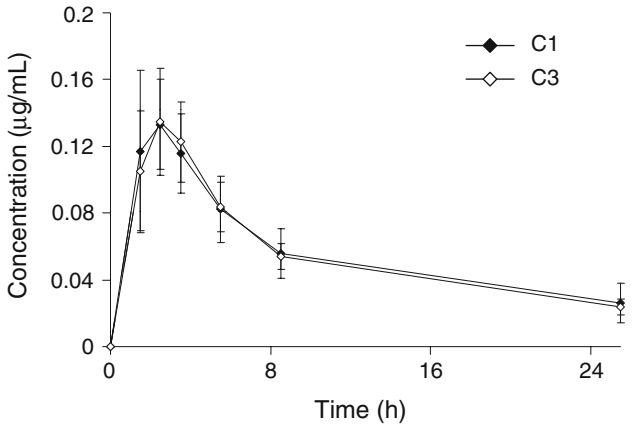

Fig. 4 Mean SN-38G concentrations versus time profiles either alone or in combination with BV were superimposed

combination with BV were also nearly superimposed (Fig. 4).

There were no significant differences in the changes in the ratio of CPT-11 to SN-38 between cycles 1 and 3 (Table 3), as well as in the ratio of SN-38 to SN-38G.

Table 3 Changes in ratio of CPT-11 to SN-38 and SN-38 to SN-38G

Patient No.	AUC ratio of SN-38/CPT-11(%)		AUC ratio of SN-38G/SN-38	
	BV (−)	BV (+)	BV (−)	BV (+)
1	3.1	4.1	3.8	3.9
2	2.2	2.2	7.9	5.9
3	2.5	1.8	8.7	8.0
4	9.2	1.8	2.3	7.7
5	0.6	0.7	23.1	22.3
6	0.3	0.3	51.7	76.0
7	4.4	1.4	3.5	8.8
8	1.0	0.5	13	23.8
9	1.0	0.8	13.5	14.6
10	5.6	4.3	2.3	3.2

There were no significant differences in the AUC ratios of SN-38/CPT-11 and SN-38G/SN-38 between cycle 1 (BV-) and cycle 3 (BV+); paired *t* test

The results indicate that the CPT-11 and BV combination had no effect on the extent of conversion of CPT-11 into its metabolites SN-38 and SN-38G.

We also observed a larger inter-patient variability for the changes in the ratios of CPT-11 to SN-38 and SN-38 to SN-38G (Table 3).

Discussion

In the present study, we found no significant differences in the mean AUCs, C_{max} and CPT-11 clearance after BV addition. Our results demonstrate that BV addition to CPT-11 (in the FOLFIRI regimen) showed no effect on the drug disposal of CPT-11 and its metabolites. This is the limited sample size study, but this is the first report clarifying the effect of BV on CPT-11 metabolism in humans.

Gaudreault et al. previously reported on the effect of BV on CPT-11 metabolism and safety using cynomolgus monkeys as subjects. Their report was the only published study available in the literature search regarding the effect of BV on CPT-11 metabolism. In their study, monkeys received bolus IFL with or without BV, and blood samples were collected for PK analysis of CPT-11 and 5-FU. They concluded that BV had no effect on the metabolism of either agent, although the number of animals tested in each group was small [with BV ($n = 5$); without BV ($n = 4$)] and no statistical comparison between groups was performed [9].

As previously shown, in the AVF2107g study, CPT-11 metabolism was characterized in a small PK study (results are presented only in the package insert of BV

[7]). In the results, SN-38 concentrations were on average 33% higher in patients receiving bolus IFL in combination with BV compared with bolus IFL alone. But it might be caused by an imbalance between the two treatment arms and a possible inter-subject variability of CPT-11 metabolism. Inter-patient variability of CPT-11 metabolism was previously reported [10], and such variability appears to be caused by inter-individual variability of carboxylesterase activity [4, 5], or glucuroconjugate activity correlated with UGT1A1 polymorphism [6]. In the present study, we could indeed observe a large inter-patient variability of CPT-11 catabolism, which is another good area for future investigation. This was not performed here since investigations into metabolic enzymes or genetic polymorphism with inter-patient comparison were not the specific aims of the present study. Here, we used intra-patient comparison to exclude inter-patient variability. As a result, we were able to clarify that BV has no effect on CPT-11 catabolism. Moreover, BV appeared to exert no effect on the conversion ratios of CPT-11 to SN-38 and SN-38 to SN-38G (Table 3). The explanation of the lacking pharmacokinetic interaction between BV and CPT-11 may be caused by different pathways of clearance: IgGs are cleared through Fc/Fc/Rn systems, whereas CPT-11 are primary enzymatically transformed in the liver [11, 12]. The analysis of PK parameters failed to provide any explanation for the observed supra-additive clinical efficacy of the CPT-11 and BV combination [2, 3]. The absence of PK interaction between CPT-11 and BV has been recognized to indicate the safety of this combination therapy for further clinical study and general practice.

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