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ORIGINAL ARTICLE

Co-administration of irinotecan decreases the plasma concentration of an active metabolite of amrubicin, amrubicinol in rats

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Abstract
Purpose This study examined the pharmacokinetics of irinotecan (CPT-11), active metabolite 7-ethyl-10-hydroxy-camptothecin (SN-38), SN-38 glucuronide (SN-38G) amrubicin (AMR), and active metabolite amrubicinol (AMR-OH) after intravenous administration of this combination therapy in rats.
Methods Male Sprague-Dawley rats were treated with 10 mg/kg CPT-11 with 10 mg/kg AMR. AMR, AMR-OH, CPT-11, SN-38 and SN-38G were measured in plasma, bile, and tissues using high-performance liquid chromatography.
Results Co-administration of CPT-11 resulted in a significant decrease in plasma concentrations and area under the curves (AUC) of AMR-OH compared with treatment with AMR alone. On the other hand, co-administration of AMR resulted in a slight increase in the initial plasma concentration of SN-38; however, there were no differences in AUC values in CPT-11 and SN-38. The cumulative biliary excretion curves of AMR, CPT-11, and their active metabolites were not changed. CPT-11 inhibited the conversion of AMR to AMR-OH in rat cytosolic fractions.
Conclusions CPT-11 did not affect the pharmacokinetic of AMR but decreased the plasma concentration of AMR-OH and might affect the formation of AMR-OH from AMR in hepatocytes.

Keywords Amrubicin · Amrubicinol · Irinotecan · Drug–drug interaction

Introduction

Amrubicin (AMR), a synthetic 9-aminoanthracyline agent, is used in Japan and is being evaluated in ongoing phase I/II studies in the US and other countries for the treatment of small-cell lung cancer (SCLC) [1, 2] and non-small-cell lung cancer (NSCLC) [3, 4]. In phase II trials of amrubicin monotherapy for lung cancer, rates of clinical response in chemotherapy-naive patients with SCLC or NSCLC were 78.8 and 24.8%, respectively. AMR is converted enzymatically to the C-13 hydroxy-metabolite amrubicinol (AMR-OH) by carbonyl reductase. AMR-OH is an active metabolite with a cytotoxicity 10–200 times that of AMR in vitro [5, 6]. AMR and AMR-OH are inhibitors of DNA topoisomerase II. AMR showed more potent antitumor activity than doxorubicin in several human tumor xenografts implanted in animal models [5, 7].

Irinotecan (CPT-11), an inhibitor of DNA topoisomerase I, is widely used in the treatment of several types of solid tumors, including lung cancer [8]. CPT-11 exerts its antitumor activity after enzymatic transformation to its more active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38), by carboxylesterases. SN-38 is subsequently conjugated in the liver by uridine diphospho-glucuronosyl transferase

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(UGT) 1A, thereby forming the inactive metabolite SN-38 glucuronide (SN-38G) [8, 9]. The major elimination route for CPT-11 and its metabolites is via biliary excretion. P-glycoprotein (P-gp), multidrug resistant-associated protein 2 (MRP2), and breast cancer resistance protein (BCRP), are responsible for the biliary excretion of CPT-11 and its metabolites [9–12].

Based on the different cellular targets (topoisomerase I for CPT-11 and topoisomerase II for AMR), both compounds are used as single-agent treatments for lung cancer. However, the current standard chemotherapy for SCLC or NSCLC is combination chemotherapy [13]. A combination study of CPT-11 and AMR was conducted to determine the recommended doses and safety in a phase II study of patients with SCLC [3, 14–17]. Yanahihara et al. [15] reported that AMR did not affect the pharmacokinetics of CPT-11 and its active metabolite SN-38, and the recommended dose of CPT-11 and AMR for phase II study. However, there are little data on the pharmacokinetics of AMR and CPT-11 when used in combination. This study was designed to explore the pharmacokinetics of CPT-11, AMR, and their active metabolites after intravenous administration of this combination therapy in rats.

Materials and methods

Chemicals and reagents

CPT-11, SN-38, and SN-38G were provided by Daiichi Sankyo Co. Ltd. (Tokyo, Japan). AMR and AMR-OH were provided by Sumitomo Pharmaceutical Co. Ltd. (Osaka, Japan). All other chemicals were commercially available products and of analytical grade.

Experimental animals

Male Sprague-Dawley rats (250–300 g) were purchased from Kyudo Co. Ltd. (Kumamoto, Japan). Rats were housed in a standard animal maintenance facility at constant temperature (21–23°C), humidity (50–70%), and a 12-h light/dark cycle for at least 1 week before the day of the experiment. All animal experiments were conducted according to the guidelines of Kumamoto University for the care and use of laboratory animals.

Pharmacokinetics of plasma and biliary excretion

To elucidate the effect of drug–drug interaction between CPT-11 and AMR, these drugs were simultaneously administered in rats. CPT-11 10 mg/kg was administered intravenously to rats via the left jugular vein immediately after the administration of 10 mg/kg AMR. 0.5 mL blood samples were collected from the right jugular vein at 5, 10, 20, and 40 min, and 1, 2, and 4 h after injection of CPT-11 with AMR. Similarly, rat receiving single AMR or CPT-11 single therapy served as controls. The plasma concentrations of parent drugs and their metabolites were determined from same animals and the blood volume taken was not replaced by the saline. Rats under anesthesia with pentobarbital (40 mg/kg) were cannulated with polyethylene tubes into the bile duct. Bile was collected from a cannula implanted in the bile duct. Samples were collected at various intervals, from the injection of drugs to 5 min, 5–10 min, 10–20 min, 20–40 min, 40 min–1 h, and more than 1 h. Blood samples were centrifuged at 900*g* for 15 min, and plasma and bile samples were stored at −80°C, respectively, until analysis. After collecting final blood samples, 0.5 g organ tissue samples, such as liver, kidney, lung, and intestine, were collected. These tissues were homogenized in ice cold 50 mM phosphate buffer (pH 7.4) and centrifuged at 1,000*g* for 10 min. The supernatants were analyzed in terms of tissue concentration. The concentrations of CPT-11, SN-38, and SN-38G were measured by high-performance liquid chromatography (HPLC) as described previously [18]. SN-38G was quantified after converting to SN-38 in the body. In addition, concentrations of AMR and AMR-OH were measured by HPLC as described previously [19]. Pharmacokinetic parameters were estimated by noncompartmental model methods using WinNonlin version 5.1 software (Pharsight, Cary, NC). The area under the plasma concentration-time curve (AUC) was calculated by using conventional linear-trapezoidal method.

Conversion of AMR to AMR-OH in hepatic cytosol

Livers were harvested from male Sprague-Dawley rats. Liver tissues were homogenized in ice cold 50 mM Tris HPLC buffer (pH 7.4) containing 150 mM KCl and centrifuged at 8,500*g* for 20 min at 4°C. The supernatant was centrifuged at 23,000*g* for 20 min at 4°C and then centrifuged at 140,000*g* for 90 min at 4°C. The supernatant liver cytosol was stored at −80°C until use. Reactions were started by adding NADPH (250 μM), and after incubation for 5 min, 100 μM reaction buffer was mixed with 100 μL of ice–cold methanol to stop the reaction.

Statistical analysis

Statistic differences between single and combination therapy were analyzed using the unpaired student *t* test. Data are shown as mean ± SE. A P value < 0.05 was considered significant.

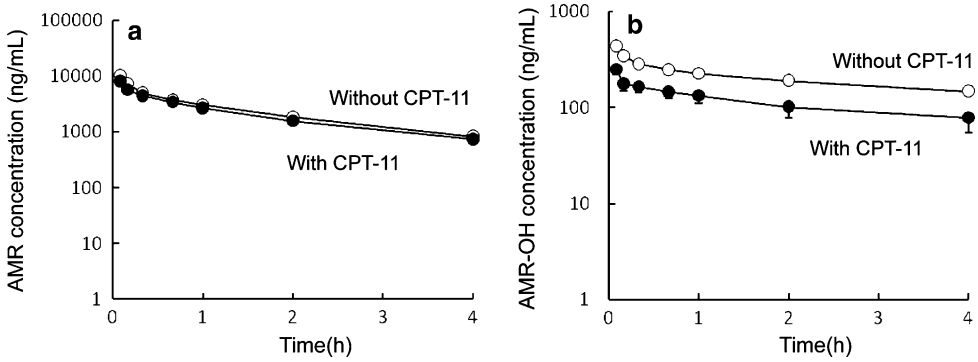

Fig. 1 Plasma concentration-time profiles of AMR (**a**) and AMR-OH (**b**) with or without CPT-11 in rats. Plasma concentration-time profiles of AMR and AMR-OH in rats after intravenous administration of AMR (10 mg/kg) without (*circle*) or with (*filled circle*) CPT-11 (10 mg/kg). Each *point* represents the mean ± SE from eight rats

Table 1 Pharmacokinetic parameters of AMR and AMR-OH with or without CPT-11 in rats. Each value represents the mean ± SE from eight rats. AUC ratio: AMR-OH/AMR AUC (%); *ns* not significance

		Without CPT-11	With CPT-11	*P*
AMR	C_{max} (μg/mL)	10.2 ± 0.76	8.3 ± 0.4	0.04
	$t_{1/2}$ (min)	101.4 ± 8.3	99.9 ± 3.0	ns
	$AUC_{0\text{-}4\ h}$ (μg/min per mL)	629.3 ± 50.8	544.6 ± 25.2	ns
	$AUC_{0\text{-}inf}$ (μg/min per mL)	755.7 ± 68.0	651.0 ± 31.6	ns
	CL (mL/min per kg)	14.1 ± 1.4	15.6 ± 0.8	ns
	Vss (mL/kg)	1,754 ± 523	1,888 ± 270	ns
AMR-OH	C_{max} (μg/mL)	0.44 ± 0.03	0.26 ± 0.03	<0.01
	$t_{1/2}$ (min)	347.5 ± 43.8	245.1 ± 80.0	ns
	$AUC_{0\text{-}4\ h}$ (μg/min per mL)	50.5 ± 4.4	28.5 ± 5.5	<0.01
	$AUC_{0\text{-}inf}$ (μg/min per mL)	128.9 ± 17.4	73.1 ± 30.3	ns
AUC ratio (%)		8.0 ± 0.4	5.3 ± 1.1	

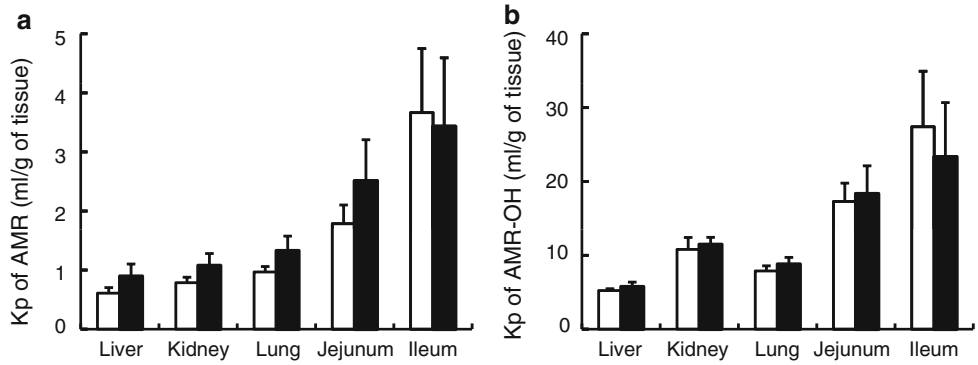

Fig. 2 Effects of CPT-11 on AMR (**a**) and AMR-OH (**b**) distribution to tissues. *K*p value of AMR and AMR-OH in rats after intravenous administration of AMR (10 mg/kg) without (*square*) or with (*filled square*) CPT-11 (10 mg/kg). Each *column* represents the mean ± SE from six rats

Results

Figure 1 shows the plasma concentration-time profiles of AMR and AMR-OH with or without CPT-11 (10 mg/kg) in rats. The C_{max} and pharmacokinetics profiles of CPT-11 and AMR in rats model after the administration of CPT-11 and AMR at a dose of 10 mg/day were nearly equal to human data [15, 19] Co-administration of CPT-11 mainly affected the pharmacokinetic parameters of AMR (Table 1). In particular, co-administration of CPT-11 resulted in a significant decrease in the plasma concentration of AMR-OH compared with treatment with AMR alone. The AUC of AMR-OH decreased by 43.4% with co-administration of CPT-11 ($P < 0.01$). The AUC, *t*1/2, CL and Vss of AMR were not changed with combination therapy. Figure 2 shows the ratio (*K*p) of plasma concentration to tissue concentration of AMR and AMR-OH with and without administration of CPT-11. There were no differences in *K*p when AMR was used alone or in combination with CPT-11. Similarly, the cumulative biliary excretion curves of AMR and AMR-OH were not changed by co-administration of CPT-11 (Fig. 3).

There were no differences in AUC, CL, and Vss values in CPT-11, SN-38, or SN-38G (Table 2, Fig. 4), and in *K*p of CPT-11, SN-38, and SN-38G when CPT-11 was used alone or in combination with AMR (data not shown). The

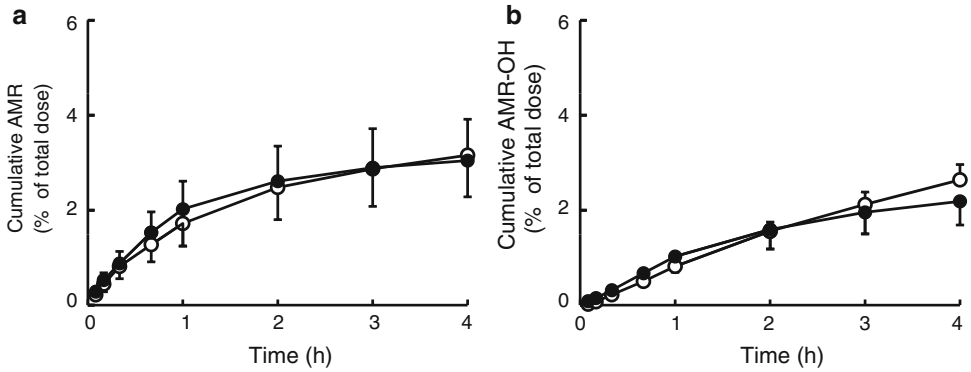

Fig. 3 Effects of CPT-11 on AMR (**a**) and AMR-OH (**b**) excretion into bile. Cumulative amounts-time profiles of AMR and AMR-OH excreted into the bile in rats after intravenous administration of AMR (10 mg/kg) without (*circle*) or with (*filled circle*) CPT-11 (10 mg/kg). Each *point* represents the mean ± SE from 5 to 6 rats

Table 2 Pharmacokinetic parameters of CPT-11, SN-38 and SN-38G with or without AMR in rats. Each value represents the mean ± SE from eight rats. *ns* not significance

		Without AMR	With AMR	*P*
CPT-11	C_{max} (μg/mL)	3.3 ± 0.2	2.9 ± 0.4	ns
	$t_{1/2}$ (min)	96.0 ± 7.8	98.2 ± 10.0	ns
	$AUC_{0\text{-}4\,h}$ (μg/min per mL)	291.1 ± 32.5	244.2 ± 44.7	ns
	$AUC_{0\text{-}inf}$ (μg/min per mL)	322.1 ± 43.3	231.0 ± 22.9	ns
	CL (mL/min per kg)	33.2 ± 3.2	38.9 ± 5.3	ns
	Vss (mL/kg)	4,046 ± 794	4,820 ± 1,564	ns
SN-38	C_{max} (μg/mL)	0.18 ± 0.02	0.33 ± 0.05	<0.05
	$t_{1/2}$ (min)	433.9 ± 233.3	156.2 ± 30.3	ns
	$AUC_{0\text{-}4\,h}$ (μg/min per mL)	21.5 ± 2.5	30.4 ± 6.1	ns
	$AUC_{0\text{-}inf}$ (μg/min per mL)	50.0 ± 9.7	38.8 ± 11.9	ns
SN-38G	C_{max} (μg/mL)	0.16 ± 0.01	0.14 ± 0.01	ns
	$t_{1/2}$ (min)	513.1 ± 144.1	340.7 ± 67.5	ns
	$AUC_{0\text{-}4\,h}$ (μg/min per mL)	22.3 ± 2.0	21.1 ± 1.9	ns
	$AUC_{0\text{-}inf}$ (μg/min per mL)	71.3 ± 12.6	48.4 ± 10.3	ns

cumulative biliary excretion curves of CPT-11 and its metabolites were not altered by AMR.

Production of AMR-OH from 20 μM AMR over a 5-min incubation period with rat liver cytosolic fraction is shown in Fig. 5. CPT-11 inhibited the conversion of AMR-OH in a dose-dependent manner, whereas SN-38 did not inhibit the conversion of AMR-OH. These data suggest that CPT-11 might inhibit the formation of AMR-OH from AMR in hepatic cytosol.

Discussion

The efficacy of combination therapy with various anticancer agents is well known, however, there is little information on the pharmacokinetic interactions of combination chemotherapy. The present data demonstrate that co-administered CPT-11 with AMR decreases the plasma concentration of AMR-OH in a rat model. The plasma concentration of AMR was not increased by CPT-11 even though the concentration of AMR-OH was decreased. We still do not know the reason why AMR was not increased but rather decreased. The possible reasons may be that the formation ratio of AMR to AMR-OH is about 5% and AMR-OH at relatively low level could be significantly affected by CPT-11 as compared to AMR. CPT-11 did not change the *t*1/2 and Vss of AMR and AMR-OH, because the conversion of AMR to AMR-OH was inhibited by CPT-11 (Fig. 1, Table 1). Therefore, CPT-11 might not affect the elimination and distribution process of AMR and AMR-OH.

On the other hand, 50 μM CPT-11 inhibited the conversion of AMR-OH from a parent drug, AMR in dose-dependent manner (Fig. 5). The *K*p values of CPT-11 and AMR in the liver after the administration of CPT-11 and AMR were 41 and 0.9 ml/g tissue, respectively; therefore, the concentration of CPT-11 in the liver tissue was about 40 times higher than that in plasma concentration. Considering the C_{max} of CPT-11 and the *K*p values of CPT-11, the concentration of CPT-11 was thought to reach more than 50 μM in the liver tissue to inhibit the conversion of AMR to AMR-OH in the rat liver cytosol. Because hepatic metabolism and biliary excretion play principal roles in the metabolism and clearance of AMR and CPT-11, this combination therapy has a potential risk of drug–drug interactions. P-gp, MRP2, and BCRP, expressed at the bile canalicular membrane,

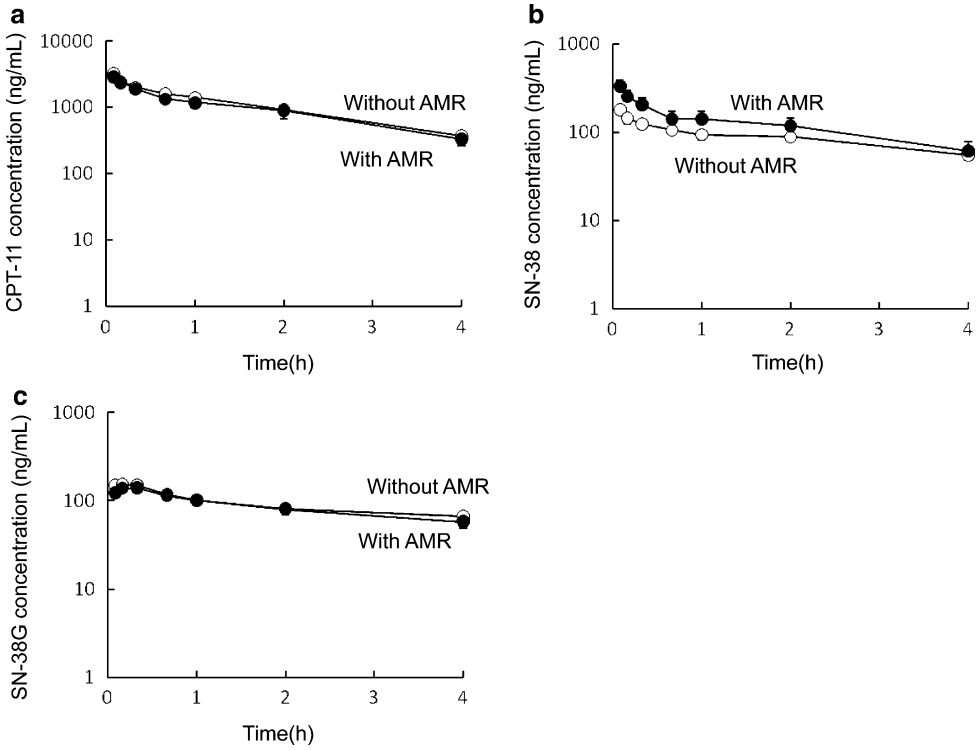

Fig. 4 Plasma concentration-time profiles of CPT-11 (**a**), SN-38 (**b**) and SN-38G (**c**) with or without AMR in rats. Plasma concentration-time profiles of CPT-11, SN-38 and SN-38G in rats after intravenous administration of CPT-11 (10 mg/kg) without (*circle*) or with (*filled circle*) AMR (10 mg/kg). Each *point* represents the mean ± SE from eight rats

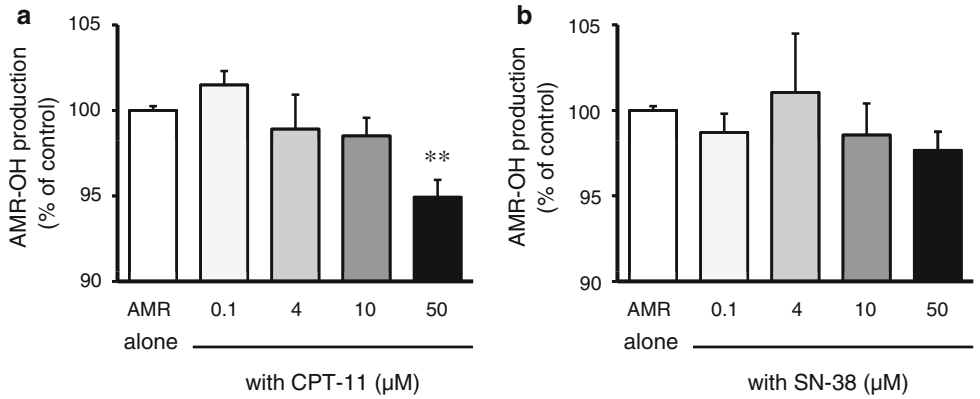

Fig. 5 Effect of CPT-11 on AMR-OH formation in cytosol fraction from rat livers. AMR-OH formation was measured by incubating 20 μM AMR in the presence of CPT-11 (**a**) or SN-38 (**b**) with erythrocyte fractions (1 mg protein/mL) in 50 mM Tris-HCl containing 150 mM KCl, pH 7.4, 37°C. Reactions were started by adding NADPH (250 μM), and AMR-OH were measured at 5 min. Each *column* represents the mean ± SE from 3 to 4 independent measurements. $^{**}p < 0.01$ versus AMR alone

appear to be responsible for the biliary excretion of AMR and AMR-OH [20]. Drug–drug interactions between AMR and CPT-11 during the biliary excretion process may be caused via P-gp. In our study, the cumulative biliary excretion of AMR and AMR-OH were not changed by co-administration of CPT-11 (Fig. 3). These results suggested that co-administration of CPT-11 did not affect the biliary excretion process of AMR and AMR-OH via transporters; however, the reason that CPT-11 did not affect the plasma concentration of AMR in rats remains unclear.

Yanaihara et al. [15] reported on a phase I study of CPT-11 and AMR in advanced NSCLC in humans. In that study, AMR did not affect the pharmacokinetics of CPT-11, SN-38, or SN-38G; however, the effect of CPT-11 on the pharmacokinetics of AMR remains unclear. They found that the AUC ratio of AMR-OH/AMR was 8% for combination treatment with AMR with CPT-11. On the other hand, the AUC ratio of AMR-OH/AMR was about 15% for AMR monotherapy in humans [21]. Recently, we also found that CPT-11 inhibited the conversion

of AMR to AMR-OH in human hepatic cytosol (data not shown), suggesting that co-administration of CPT-11 affect pharmacokinetics of AMR-OH. Taken together, the present results suggested that drug–drug interaction between AMR and CPT-11 might be observed in not only rat model but human.

Co-administration of CPT-11 decreased the plasma concentration of AMR-OH and did not change the plasma concentration of AMR, raising the possibility that reduced plasma concentration of AMR-OH may affect the antitumor activity. In part, the mechanism of this drug–drug interaction may be inhibitory effect of CPT-11 on the formation of AMR-OH in hepatic cytosol. An active metabolite SN-38 does not affect the formation of AMR-OH in vitro (Fig. 5), however, the reason for the differential effect of CPT-11 and SN-38 on the formation of AMR to AMR-OH and the pharmacokinetics of AMR and AMR-OH is unclear. This differential inhibitory effect of SN-38 and CPT-11 on the formation of AMR-OH may be caused by the degree of different efficacy of protein binding in vivo. The plasma bindings of CPT-11 and SN-38 were reported to be about 60 and 95%, respectively [21]. Since the free-plasma concentration of CPT-11 is much higher than that of SN-38, CPT-11 may inhibit the formation of AMR-OH in a dose-dependent manner, whereas SN-38 does not inhibit it. As for other reason, SN-38G or the metabolite of CPT-11 may inhibit the formation of AMR-OH.

We did not demonstrate whether the inhibitory effect of CPT-11 on the formation of AMR-OH affect the antitumor efficacy of AMR. Studies examining the best dosing schedule for both agents in terms of both pharmacokinetic reactions and clinical effects are important when exploring combination chemotherapy using AMR. The best administration schedule for combination chemotherapy to achieve the highest clinical benefit needs to be determined. For example, we should consider simultaneous treatment as well as a sequential treatment to elucidate the pharmacokinetic interaction between AMR and CPT-11. However, because of the considerable differences in the inhibitory effects of CPT-11 on enzymatic conversion of AMR to AMR-OH between humans [15] and rats in the present data, we cannot assume that findings from animal experiments will be true in humans and the possible pharmacokinetics interaction proposed current experiments should be tested in clinical studies. Further studies are needed to confirm possible drugdrug interactions and the most effective regimen for combination therapy based on pharmacokinetic data.

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Conflict of interest statement Authors indicated no potential conflicts of interest.

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