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ORIGINAL ARTICLE

Pharmacokinetic and pharmacodynamic study on amrubicin and amrubicinol in Japanese patients with lung cancer

Yoshinori Makino · Noboru Yamamoto · Hitoshi Sato · Reiko Ando · Yasushi Goto · Chiharu Tanai · Hajime Asahina · Hiroshi Nokihara · Ikuo Sekine · Hideo Kunitoh · Yuichiro Ohe · Erika Sugiyama · Nobuaki Yokote · Tomohide Tamura · Hiroshi Yamamoto

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Abstract
Purpose The pharmacokinetic (PK)–pharmacodynamic (PD) relationship of amrubicin and its active metabolite, amrubicinol, has only been evaluated using trough levels of these agents since the full PK profiles not yet been clarified so far. This study was performed to analyze the full PK profiles of amrubicin and amrubicinol and to evaluate their toxicity–PK relationships in Japanese patients.
Methods Amrubicin (35–40 mg/m^2) was administered to 21 lung cancer patients on days 1–3 every 3–4 weeks. Fourteen blood samples were obtained per patient over the course of 3 administration days. The plasma concentrations of amrubicin and amrubicinol were quantitated by HPLC, and the relationships between PK parameters of these compounds and hematological toxicities were evaluated.
Results The overall PK profiles of amrubicin and amrubicinol were well characterized using a 3-compartment model and a 1-compartment model with a first-order metabolic process, respectively. The major toxicities were hematological. The clearance of amrubicinol was significantly correlated with grade 4 neutropenia ($P = 0.01$). The percentage decreases in the neutrophil count, hemoglobin level and platelet count were well correlated with the amrubicinol AUC.
Conclusion The pharmacokinetic profiles of amrubicin and amrubicinol were clarified, and the subsequent PK–PD analyses indicate that the clearance of amrubicinol is the major determinant of neutropenia.

Keywords Amrubicin · Amrubicinol · PK–PD study · Myelosuppression · Blood cell destruction

Y. Makino (✉) · R. Ando · N. Yokote · H. Yamamoto
Department of Pharmacy, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
e-mail: ymakino@ncc.go.jp

N. Yamamoto · Y. Goto · C. Tanai · H. Asahina · H. Nokihara · I. Sekine · H. Kunitoh · Y. Ohe · T. Tamura
Division of Internal Medicine and Thoracic Oncology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

H. Sato · E. Sugiyama
Clinical and Molecular Pharmacokinetics/Pharmacodynamics, School of Pharmaceutical Sciences, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan

Introduction

Amrubicin (AMR) and its active metabolite, amrubicinol (AMR-OH), markedly inhibit topoisomerase II activity and are effective against lung cancer [1]. AMR is approved in Japan for the treatment of small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC). In AMR monotherapy, AMR is administered at a dose of 35–45 mg/m^2/day on three consecutive days every 3–4 weeks. Six phase II studies for second-line or third-line AMR monotherapy for the treatment of SCLC have demonstrated overall response rates (ORRs) of 21–53% and a median survival period of 6–12 months [2–7]. Two phase II studies of previously treated NSCLC have been reported, with ORRs of 11.5 and 13.5%, respectively [5, 8]. In these phase II studies, the incidences of grade 3 or 4 myelosuppression were 82% (39–97%) [Median (Range)] for neutropenia, 28% (8–38%) for thrombocytopenia and 27% (5–41%) for anemia, respectively. Furthermore, the incidence of febrile neutropenia was 12% (2–35%).

The pharmacokinetic (PK)–pharmacodynamic (PD) profiles of AMR and AMR-OH have not yet been fully clarified so far. In a previous report by Matsunaga et al. [9],

full-sampling data were obtained from only one subject treated with 30 mg/m^2 of amrubicin and other data were obtained spars-sampling points from 15 patients (30–45 mg/m^2). On the other hand, significant relationships were observed between the plasma trough level of AMR-OH on day 4 and neutropenia and anemia [10]. The trough level of AMR-OH was correlated with the percent change in the neutrophil count [10]. However, the previous studies did not obtain plasma sampling points capable of fully characterizing the PK profiles of AMR and AMR-OH for consecutive days. Generally, the use of plasma drug concentration at only one particular time point should cause some uncertainty for establishing efficacy–PK or toxicity–PK relationships, and full PK profiling of a drug and subsequent modeling approach are highly preferable.

We, therefore, conducted a PK–PD study on AMR and AMR-OH in which we determined the PK model parameters of these agents throughout 3 days of administration and evaluated the toxicity–PK relationships in Japanese lung cancer patients based on the full PK profiles.

Materials and methods

Patients and treatments

This study was conducted at the National Cancer Center Hospital, Tokyo, Japan. Patients were eligible for participation in this study if they were 20 years or older and had been diagnosed as having lung cancer and had received AMR monotherapy. Patients with hepatitis B or C virus or human immunodeficiency virus infections and those who were considered by their physician to be ineligible as a trial candidate were excluded. Written informed consent was provided by each patient before study enrollment. The study was approved by the ethical review boards of the National Cancer Center Hospital and Showa University. It was conducted in accordance with the Declaration of Helsinki and all applicable laws and regulations.

AMR (Calsed^{TM}; Dainippon Sumitomo Pharmaceuticals Co., Ltd., Osaka, Japan) was dissolved in 50 mL of physiological saline and was administered intravenously as a 5-min infusion at a dose of 35–40 mg/m^2/day on days 1–3 every 3–4 weeks. For second- or later-line treatment of small or non-small-cell lung cancer with AMR, the recommended dose of AMR is 40 mg/m^2, in general, with some dose reduction (e.g., 35 mg/m^2) as needed based on the judgment of the attending physician. Prophylactic antiemetics (granisetron and dexamethasone) were used only as required and according to the physician's discretion. Other medications for underlying diseases, complications and pain control were allowed.

Before treatment, all patients underwent a medical history survey, physical and hematological examinations and serum biochemistry tests. The physical examination and biochemistry tests were repeated as part of normal clinical practice. The toxicities were graded according to the National Cancer Institute Common Toxicity Criteria, Version 3.0. Response was assessed according to the Response Evaluation Criteria in Solid Tumors [11].

Pharmacokinetic sampling and drug assays

PK evaluations were performed in all patients during the initial cycle of treatment. Heparinized venous blood samples (4 mL) were taken before infusion, at the end of the AMR infusion (0 min), as well as at 5, 15 and 30 min and 1, 2, 4, 8 and 24 h after the end of the infusion and at 0 min and 8 h after the infusions on days 2 and 3.

The plasma samples were stored at −80°C until analysis. The plasma concentrations of AMR and AMR-OH were measured using a previously reported high-performance liquid chromatography (HPLC) method [12]. The components were separated using HPLC on two reverse-phase columns linked with a connector (Onyx Monolithic C18, 100 × 4.6 mm) using 4 mM sodium 1-octanesulfonate, 2.3 mM acetic acid:tetrahydrofuran:dioxane (15:2:6, v/v/v) as an eluent. AMR and AMR-OH were measured using a fluorescence detector set at an excitation wavelength of 480 nm and a detection wavelength of 550 nm. Standard AMR and AMR-OH powders with purities >99% were supplied by Dainippon Sumitomo Pharmaceuticals Co., Ltd. (Osaka, Japan). The assay was validated according to the guidelines recommended by the U.S. Food and Drug Administration. The limit of quantitation was 2.5 ng/mL for both AMR and AMR-OH. The percentage recovery from the plasma proved to be higher than 88.1%. Intraday accuracy ranged from −4.1 to 0.8% for AMR and −9.8 to −2.1% for AMR-OH. The interday accuracy ranged from −3.1 to 3.0% for AMR and −4.0 to 2.3% for AMR-OH. The intraday precision ranged from 1.4 to 8.8% for AMR and 1.3 to 4.2% for AMR-OH. The interday precision ranged from 2.7 to 8.8% for AMR and 5.3 to 5.5% for AMR-OH.

Pharmacokinetic analysis

The PK parameters were estimated using a non-linear least-squares regression analysis (WinNonlin, Version 5.0.1; Pharsight, Cary, NC, USA) with a weighting factor of $1/Y^2$, where Y represents the observed data. The individual plasma concentration–time data were fitted to one-, two- or three-exponential equations using a constant infusion input for AMR and a one- or two-compartment model with a first-order metabolic process from AMR to AMR-OH

(parameterized by k_{in}). The PK model was optimized on the basis of Akaike's information criteria (AIC). Fitted parameters were permitted in the computation of the following PK parameters: AUC, peak plasma concentration of day 1 (C_{max}), total body clearance (CL) and volume of distribution at steady state (Vd_{ss}).

Pharmacodynamic analysis

The relationships between PK parameters (AUC and C_{max}) of AMR-OH and the hematologic toxicity were evaluated. The percentage decrease in the hematologic count or level (neutrophils and hemoglobin) was calculated as follows:

$$\%\,\text{Decrease in hematologic count or level} = \frac{\text{pretreatment count or level} - \text{nadir count or level}}{\text{pretreatment count or level}} \times 100$$

The results were plotted as a function of the AUC and C_{max} of AMR-OH, respectively. Relationships between adverse effects and PK exposure (AUC) or C_{max} were fitted using a non-linear least-squares regression and a weighting factor of unity according to a sigmoid model, as follows:

$$\text{Adverse effect } (\%) = \frac{E_{max} \cdot \text{AUC}^{\gamma}}{\text{EC}_{50}{}^{\gamma} + \text{AUC}^{\gamma}} \times 100$$

or

$$\text{Adverse effect } (\%) = \frac{E_{max} \cdot C_{max}{}^{\gamma}}{\text{EC}_{50}{}^{\gamma} + C_{max}{}^{\gamma}} \times 100$$

where E_{max} represents the maximum effect and EC_{50} is the AUC (or C_{max}) of AMR-OH at which the effect was 50% of the maximum effect. A non-linear least-squares regression was conducted using WinNonlin to estimate E_{max}, EC_{50} and the sigmoidicity coefficient (γ). The strength of the relationship between the percent decrease in the hemoglobin level and the AUC (or C_{max}) of AMR-OH was assessed using a least-squares linear regression analysis.

In the PD analysis, the patient characteristics as well as the PK parameters were compared among patients who experienced grade 4 neutropenia (<500/μL). The patient characteristics that were evaluated for possible association with grade 4 neutropenia were age, sex, performance status (0 vs. ≥1), type of disease (SCLC vs. NSCLC), smoking index, prior surgery, prior thoracic or brain irradiation, the number of prior chemotherapy regimens (1 vs. ≥2), albumin, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), serum α_1-acid glycoprotein (AGP) and pretreatment neutrophil counts. PK parameters, including C_{max}, AUC and CL of AMR and AMR-OH, were also compared between patients who experienced and those who did not experience grade 4 neutropenia.

Statistical analyses

The obtained data were presented as mean ± standard deviation (SD). To identify factors associated with grade 4 neutropenia, continuous variables were compared between patients with and those without grade 4 neutropenia using the Mann–Whitney *U*-test, and differences in the distribution of dichotomized variables were evaluated using the χ^2-test or the Fisher exact test, as appropriate. $P < 0.05$ was considered statistically significant, and all *P*-values were two-tailed. To identify variables significantly associated with grade 4 neutropenia, multivariate logistic regression analyses were performed. All statistical analyses were performed using the statistical software JMP 4.0 (SAS Institute, Cary, NC, USA).

Results

Patient characteristics

Twenty-one patients were enrolled in this study from May 2007 to June 2009. The patient characteristics are listed in Table 1. Seventeen patients had SCLC, and 4 patients had NSCLC. Seventeen patients were men, and 4 were women; all patients had a good performance status, and the median age was 64 years. All 21 patients had previously undergone at least one chemotherapy regimen. All patients had received a platinum agent (cisplatin or carboplatin), and 20 patients had received some form of topoisomerase inhibitor (irinotecan, etoposide or topotecan). Only one patient who had been diagnosed as having squamous cell carcinoma had not been treated with a topoisomerase inhibitor. All patients were included in the PK and toxicity evaluations. Eighteen patients were assessed for response and survival. Two patients were not assessed for response because of the occurrence of interstitial pneumonia or a cardiac event after the second cycle; chemotherapy was discontinued in these patients. Another patient developed disseminated intravascular coagulation (DIC), and chemotherapy was ceased because of the presence of grade 4 thrombocytopenia despite an insufficient response during the first cycle. Seven patients had received granulocyte colony-stimulating factor during the first cycle. All 21 patients had received prophylactic antiemetics. Among these patients, 8 were treated with granisetron and dexamethasone and 13 were treated with granisetron only prior to treatment with AMR on 3 consecutive days.

Pharmacokinetics

Patients received AMR at a dose of 40 mg/m^2 except for one patient with squamous cell carcinoma who received a

Table 1 Patient characteristics

$n = 21$	n	Median	Range
Sex			
Male/female	17/4		
Age (y.o.)		64	39–81
Disease			
SCLC (LD/ED)	5/12		
NSCLC (LCNEC/SQ)	3/1		
PS			
0/1/2	10/10/1		
Smoking history			
±	2/19		
Smoking index		1,165	0–3,200
Pretreatment			
Surgery			
±	15/6		
Radiation			
±	11/10		
Thoracic	5		
Whole brain	6		
Other	1		
Chemotherapy			
0/1/2≤	0/16/5		
CDDP/CPT	11		
CBDCA/ETOP	8		
Others	12		
Characteristics			
Height (cm)		163.8	155–177.5
Body weight (kg)		57	36–74.95
Body surface area (m^2)		1.62	1.28–1.89
Serum creatinine (mg/dL)		0.8	0.6–1.5
Aspartate amino transferase, AST (IU/L)		24	15–111
Alanine transaminase, ALT (IU/L)		18	7–61
Total bilirubin (mg/dL)		0.4	0.3–1.5
Lactate dehydrogenase, LDH (U/L)		233	133–1,286
Serum albumin (g/dL)		3.9	2.5–4.6
α_1-Acid glycoprotein, AGP (mg/dL)[a]		103.5	50–292
White blood cell (×1,000/μL)		5.4	2.5–15.2
Hemoglobin (g/dL)		11.9	7.2–15.3
Platelet (×10,000/μL)		22.2	12.2–37.3
Absolute neutrophil count, ANC (×1,000/μL)		3.6	1.4–11.9

SCLC small-cell lung cancer, *LD* limited disease, *ED* extensive disease, *NSCLC* non-small-cell lung cancer, *LCNEC* large-cell neuroendocrine carcinoma, *SQ* squamous cell carcinoma, *PS* performance status, *CDDP* cisplatin, *CPT* irinotecan, *CBDCA* carboplatin, *ETOP* etoposide

[a] α_1-Acid glycoprotein (AGP) data were obtained from 18 patients

dose of 35 mg/m^2 based on the judgment of the attending physician. Together, the patients received a total of 71 cycles (median of 4 cycles [range, 1–7]) of therapy. A total of 294 plasma samples were obtained for the PK analyses. The PK profiles for AMR and AMR-OH were well characterized using a 3-compartment model and a 1-compartment model with a first-order metabolic process from AMR to AMR-OH, respectively (Fig. 1). The plasma dispositions of AMR and AMR-OH, to which the PK models were fitted, are shown in Fig. 2, and the pharmacokinetic parameters are listed in Table 2.

The plasma concentrations of AMR decreased sharply shortly after the drug infusion and then slowly declined as a result of drug elimination and distribution into the peripheral and blood compartments. The infusion of AMR was followed 2 h later (t_{max}) by the peak plasma concentration of AMR-OH (C_{max}: 23 ± 7 μg/L; Fig. 2; Table 2). In two patients, AMR was metabolized rapidly to AMR-OH, leading to the generation of a large variation in the metabolic rate constant (k_{in}).

Toxicities

Grade 3/4 hematological toxicities consisted of neutropenia (81%), thrombocytopenia (33%) and anemia (19%). The most frequent grade 4 hematological toxicity, observed in 13 patients (62%), was neutropenia. The mean percentages of the decrease in the white blood cell count, absolute neutrophil count, platelet count and hemoglobin level for all 21 patients were 71 ± 20%, 85 ± 21%, 56 ± 29% and 16 ± 7%, respectively. All non-hematological toxicities were mild (≤grade 2). Three patients (14%) experienced febrile neutropenia. Dose reduction was required in 32% (6/19) of the patients, and a treatment delay (4 weeks or more) was needed in 12 patients because of prolonged hematological toxicity during the second cycle.

Responses

One patient with SCLC had a complete response, 8 patients had partial responses (SCLC 6, large-cell neuroendocrine carcinoma 2), 4 patients had stable disease (SCLC) and 5 patients had progressive disease (SCLC 4, squamous 1).

PK–PD relationship for hematologic toxicity

The present PK–PD analyses demonstrated that the percentage decrease in the absolute neutrophil count was related to the AUC and C_{max} of AMR-OH, as described by the sigmoid maximum effect (E_{max}) model. The plot in Fig. 3a and b depicts the E_{max} relationship using the data obtained from all patients. On the basis of the E_{max} model

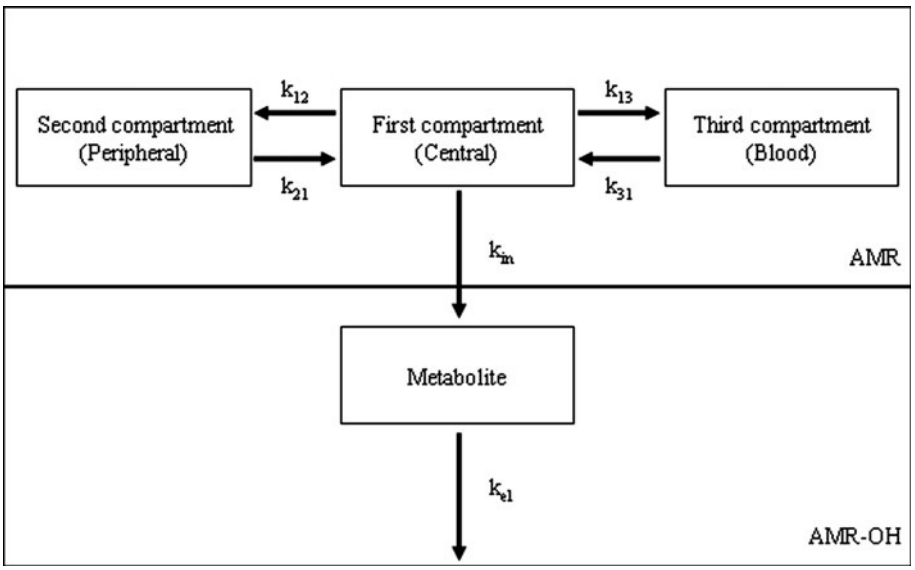

Fig. 1 Schematic illustration of pharmacokinetic model for amrubicin and amrubicinol. The model includes three compartments for amrubicin and one for amrubicinol: k_{12}, k_{21}, k_{13} and k_{31} represent the rate constants for the intercompartmental transfers of AMR. k_{in} represents the metabolic conversion rate constant from AMR to AMR-OH. k_{el} represents the elimination rate constant for AMR-OH

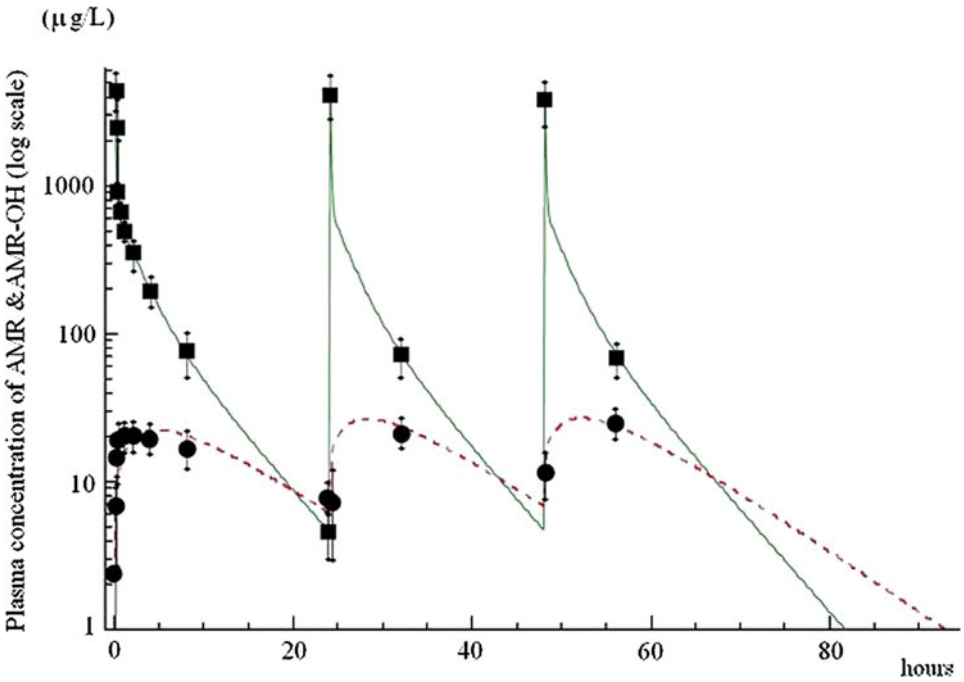

Fig. 2 Concentrations in plasma versus time curves of AMR and AMR-OH. Plasma concentration–time profiles for AMR (*filled square*) and AMR-OH (*filled circle*). *Squares* or *circles* and vertical bars, mean measured concentration ± SD; lines, best-fit lines from the pharmacokinetic analysis (*solid lines* for AMR and *dashed lines* for AMR-OH)

fitting, the AUC and C_{max} that yielded a 50% decrease in the absolute neutrophil count were predicted to be 306.1 h μg/L and 13.2 μg/L, respectively. The shapes of the curves were steep ($\gamma = 8.4$ for AUC; $\gamma = 7.7$ for C_{max}), and these models provided a correlation between the PK of AMR-OH and neutropenia ($r = 0.8296$ for AUC; $r = 0.8035$ for C_{max}). A least-squares linear regression analysis showed that the percent decrease in the hemoglobin level could be estimated by the AUC and C_{max} of AMR-OH ($r = 0.6554$ for AUC; $r = 0.7267$ for C_{max}) (Fig. 3c, d). The AUC and C_{max} that yielded a 50% decrease in the platelet count were predicted to be 549.4 h μg/L and 22.1 μg/L, respectively. The shapes of the curves were gentle ($\gamma = 2.0$ for AUC; $\gamma = 1.9$ for C_{max}), and these models provided the correlation between the PK of AMR-OH and thrombocytopenia ($r = 0.679$ for AUC; $r = 0.6301$ for C_{max}) (Fig. 3e, f). When the characteristics of patients who experienced or did not experience grade 4 neutropenia were compared, the distribution of the performance status was significantly different, and the pretreatment body weight in patients with grade 4 neutropenia was significantly lower than in those without

Table 2 Pharmacokinetic parameters of AMR and AMR-OH

PK parameter	NCA		Model (single dose)[a]		Model (multiple dose)[b]	
	Mean	SD	Mean	SD	Mean	SD
AMR (NCA; constant infusion, Model; 3-compartment infusion)						
AUC (h μg/L)	3,218	684.6	3,175	730.7	3091	579
CL (L/h)	20.6	5.1	20.1	5	21.0	4.4
C_{max} (μg/L)	4,355	1,308	5,500	3,734	4,200	1,149
Vd_{ss} (L)	71.5	15.9	73	17.1	74.0	16.8
k_{12} (1/hr)	–	–	8.0	3.0	6.4	3.5
k_{13} (1/hr)	–	–	2.0	2.7	1.0	2.1
k_{21} (1/hr)	–	–	2.4	1.8	2.4	2.5
k_{31} (1/h)	–	–	0.4	0.2	0.4	0.2
AMR-OH (NCA; extravascular, model; 1-compartment with first-order metabolism)						
k_{in} (1/h)	–	–	12.7	32.3	2.3	2.0
AUC (h μg/L)	515	146	506.9	140.1	459.5	122.3
CL (L/h)	134.3	48.2	135.3	44.9	150.0	53.9
C_{max} (μg/L)	22.5	6.7	22.2	6.6	23.1	6.7
Vd_{ss} (L)	2,899	1,083	2,935	1,223	2,708	1,042

NCA non-compartmental analysis

[a] Model analysis using 9 plasma sampling points (0, 5, 15 and 30 min, and 1, 2, 4, 8 and 24 h after the end of infusion) after AMR infusion on day 1

[b] Model analysis using all plasma sampling points (0, 5, 15 and 30 min and 1, 2, 4, 8 and 24 h after the end of infusion on day 1 and 0 min and 8 h after the end of infusion on days 2 and 3) after AMR infusion on days 1–3

(Table 3). Among the PK parameters, the CL of AMR-OH and the Vd_{ss} of AMR were significantly lower in patients with grade 4 neutropenia.

On the other hand, in the present multivariate analysis, none of the parameters were identified as significant variables of grade 4 neutropenia.

Discussion

The intrinsic activity of the metabolite of AMR, AMR-OH, has been known to be 10–100 times higher than that of AMR [13].

The aims of the present study were to determine the PK parameters of AMR and AMR-OH and to clarify the relationships between PK parameters and toxicity associated with AMR therapy. For this purpose, we carried out an extensive PK–PD study on AMR and AMR-OH in 21 patients with lung cancer, where a total of 14 blood sampling per patient was accomplished over the course of 3 intravenous AMR administration days.

The PK profiles of AMR and AMR-OH were well characterized using a 3-compartment model with a short infusion and a 1-compartment model with a first-order metabolic process from AMR to AMR-OH, respectively. All PK model parameters of AMR and AMR-OH over the 3 administration days could be well extrapolated using the compartment model parameters obtained from a 24-h single-dose and non-compartmental analysis. The PK profiles for AMR and AMR-OH did not show non-linearity or accumulation. Therefore, the 3-day PK profile can be simulated using the plasma trough level observed on the first administration day, enabling the doses on days 2 and 3 to be adjusted, if necessary.

Using the compartment analysis, we were able to perform a kinetic approach to identifying the mechanisms responsible for the metabolism of AMR to AMR-OH and the subsequent metabolic pathway, thereby enabling a quantitative correlation between the PK and the hematological toxicities arising from AMR therapy. The AMR-OH clearance is an apparent clearance, since the percentage of AMR metabolized into AMR-OH is unknown and subject to interindividual variability.

In the present PK–PD study, it was found that a higher C_{max} and AUC of AMR-OH in the plasma was associated with a risk of grade 4 neutropenia and the percentage decrease in the absolute neutrophil count, as well as with the decrease in the platelet count. On the other hand, both parameters were well correlated with a linear model of the percentage decrease in the hemoglobin level. AMR is a quinone-containing anthracycline agent. Doxorubicin has a similar quinone structure and is known to reduce its respective semiquinone-free radicals in the presence of flavoenzymes. Free radicals can also be formed from the

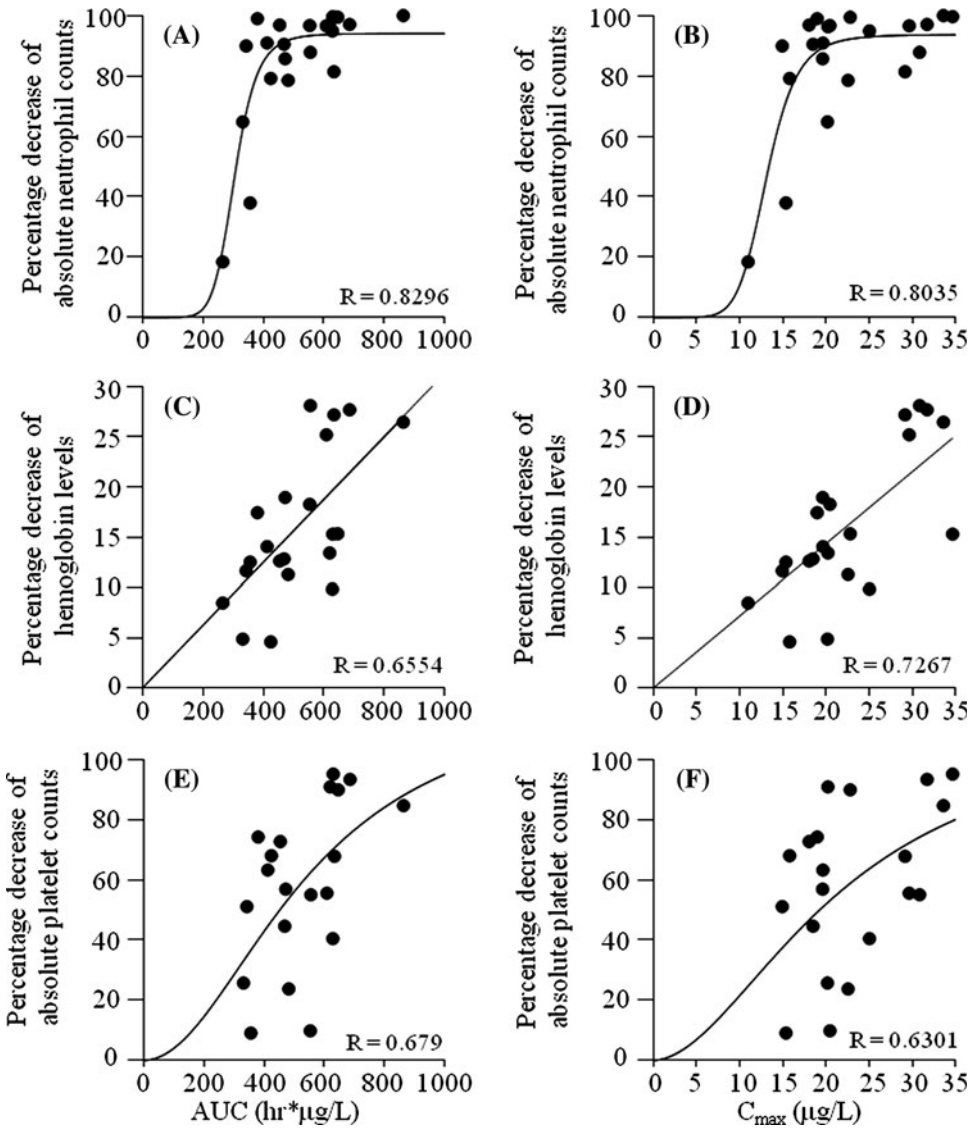

Fig. 3 PK–PD correlation between hematological toxicity and AMR-OH PK parameters. Relationship between the percent decrease in the neutrophil, hemoglobin or platelet count and the AUC or C_{max} of AMR-OH; The solid lines indicate the best fit of a sigmoid E_{max} pharmacodynamic model to the data [neutrophil, AUC: (**a**), C_{max}: (**b**), platelet, AUC: (**e**), C_{max}: (**f**)]. The *solid line* is the linear regression line, and the *dashed line* is the 95% CI for individual estimates [hemoglobin, AUC: (**c**), C_{max}: (**d**)]

interaction of doxorubicin with iron to form a doxorubicin-iron III complex. The in vitro data indicated that dexrazoxane, which prevents anthracycline-mediated cardiotoxicity, inhibited the binding of doxorubicin to red blood cells but had no effect on the association of doxorubicin with erythrocyte ghosts [14]. These findings and a previous report describing an interaction between anthracyclines and iron or hemoglobin support our notion that the third compartment of the parental compound corresponds to the blood cells and that AMR-OH was converted from AMR in the blood, forming an AMR-OH-iron III complex that may directly destroy blood cells. Therefore, the cause of the severe hematological toxicities of AMR may be related not only to myelosuppression but also to the destruction of blood cells.

In multivariate analysis, we could not find the pretreatment factors or PK parameters to predict the severe neutropenia, possibly because the sample size was too small. On the other hand, body weight was found to be positively correlated with the Vd_{ss} of AMR. Moreover, the low Vd_{ss} of AMR was significantly correlated with the high AUC of AMR-OH, suggesting that patients with the low Vd_{ss} of AMR rapidly metabolized AMR to AMR-OH; as a result, hematological toxicities tended to be serious to such patients.

AMR was metabolized rapidly to AMR-OH in 2 patients, leading to the generation of large variations in the metabolic rate constant (k_{in}). The AMR-OH concentrations at the end of infusion in these patients were 29.9 and 11.0 ng/mL, far above the average AMR-OH concentration at the corresponding time point. Other PK parameters and their variations were similar between the single-dose and multiple-dose studies. When these data were excluded, the k_{in} in the single-dose study was determined to be 2.8 ± 0.5 (mean $\pm$ SD), which is close to the k_{in} value determined in the multiple-dose study.

The major pathway of AMR metabolism involves the reduction in the C-13 carbonyl group to a hydroxyl group

Table 3 Characteristics and pharmacokinetics of AMR and AMR-OH in patients with or without grade 4 neutropenia

Neutropenia	Neutrophils ≥ 500/μL	Neutrophils < 500/μL	*P* value
Patients (*n*)	8	13	
Sex (*n*)			0.13
Female	0	4	
Male	8	9	
Performance status (n)			0.001
0	8	3	
≥1	0	10	
Number of prior chemotherapy regimens (*n*)			0.33
1	5	11	
≥2	3	2	
Age (years)			1.00
Median	65	64	
Range	39–76	42–81	
Body weight			0.02
Median	65	53	
Range	55–75	36–72	
Serum creatinine			0.11
Median	0.9	0.8	
Range	0.7–1.5	0.0–1.1	
Total bilirubin			0.19
Median	0.4	0.6	
Range	0.3–0.6	0.3–1.5	
Aspartate amino transferase, AST			0.25
Median	23	26	
Range	15–32	16–111	
Serum albumin			0.08
Median	4.1	3.8	
Range	3.5–4.4	2.5–4.6	
AMR clearance, AMR-CL (L/h)			0.06
Median	21.9	18.5	
Range	18.8–33.8	11.7–24.4	
AMR distribution, Volume at steady state, Vd_{ss} (L)			0.02
Median	79.2	63.2	
Range	64.5–116.9	45.7–87.6	
AMR-OH clearance, AMR-OH-CL (L/h)			0.01
Median	167.0	105.9	
Range	86.8–278.4	77.6–149.8	

Sex, performance status and number of prior chemotherapy regimens were analyzed using the χ^2-test or the Fisher exact test. Others were analyzed using the Mann–Whitney *U*-test

by carbonyl reductase (CBR). Then, AMR and AMR-OH are inactivated by NAD (P) H: quinone oxide reductase (NQO) and NADPH P-450 reductase [15].

Genetic polymorphisms of these metabolic enzymes are reportedly related to the PK of several anticancer agents [16–18]. CBR-1 D2 diplotypes tagged by at least one variant allele at the CBR-1 c.627C > T and +967G > A loci are correlated with significantly higher exposure levels of doxorubicin, suggesting the possibility that the intracellular conversion to doxorubicinol is reduced in Asian patients with breast cancer [15]. In another investigation, the NQO-1 609 C > T polymorphism resulted in a significantly reduced tumor NQO-1 activity and a reduced survival in subsets of patients receiving intraperitoneal hyperthermic mitomycin C [18]. However, the exact reason for interindividual variations in the PK of AMR remains unknown; thus, the relationship between genetic polymorphisms of metabolic enzymes and transporters for AMR and AMR-OH should be evaluated in the future.

In this study, several types of lung cancer patients were enrolled (including 17 patients with SCLC, 3 with large-cell neuroendocrine carcinomas and 1 with squamous cell carcinoma). Furthermore, among 17 patients with SCLC, five subjects had limited disease and 12 had extensive disease. These variations of the tumor properties inevitably led to a limitation of this study, i.e., the difficulty in clarifying the PK–PD relationship between the AUC of AMR/AMR-OH and the tumor response.

In conclusion, we clarified the full PK profiles of AMR and AMR-OH and found that the CL of AMR-OH is the major determinant of neutropenia. PK–PD evidence has not yet been reported for this compound on a global basis so far, since AMR is approved only in Japan. Thus, the present findings should be of great importance for avoiding or reducing severe hematological toxicities associated with AMR therapy. In order to confirm our findings and to identify factors influencing the interindividual variabilities in the PK–PD parameters for AMR, further population PK studies and pharmacogenetic studies on a larger number of patients are highly warranted.

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