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Evaluation for pharmacokinetic exposure of cytotoxic anticancer drugs in elderly patients receiving (R-)CHOP therapy

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(R-)miniCHOP therapy, which delivers approximately half-doses of the (R-)CHOP regimen, has shown efficacy and safety in patients who are more than 80 years old. This study aimed to compare the area under the plasma concentration–time curves (AUCs) of vincristine (VCR), doxorubicin (DXR), and cyclophosphamide (CPA) between (R-)CHOP and (R-)miniCHOP regimens. The AUCs were compared between patients aged 65–79 years receiving (R-)CHOP therapy and those aged 80 years and older receiving (R-)miniCHOP therapy. Age was not an independent variable for predicting the dose-adjusted AUCs (AUC/Ds) of cytotoxic anticancer drugs. The median AUCs of DXR and CPA were significantly smaller in the (R-)miniCHOP group than in the (R-)CHOP group (168.7 vs. 257.9 ng h/mL, $P = 0.003$, and 219.9 vs. 301.7 $\mu\text{g h/mL}$, $P = 0.020$, respectively). The median AUCs of VCR showed the same trend but the difference was not significant (24.83 vs. 34.85 ng h/mL, $P = 0.135$). It is possible that the AUCs of VCR, DXR, and CPA in patients aged 80 years and older receiving (R-)miniCHOP therapy may be lower than those in patients 65–79 years old receiving (R-)CHOP therapy.

Diffuse large B-cell lymphoma (DLBCL) accounts for more than 30% of all non-Hodgkin's lymphoma (NHL) cases in Japan¹, and its incidence continues to increase². CHOP therapy, a multidrug combination chemotherapy with vincristine (VCR), doxorubicin (DXR), cyclophosphamide (CPA), and prednisolone (PSL), is the standard chemotherapy for the treatment of various subtypes of NHL, including DLBCL. In 2002, the anti-CD20 monoclonal antibody rituximab became available for use in combination with CHOP therapy (termed R-CHOP therapy), which results in a higher survival rate than CHOP therapy alone^{3–5}. Presently, R-CHOP is the standard treatment for DLBCL, and this approach will likely continue for some time.

(R-)CHOP therapy has a variety of side effects, such as peripheral neuropathy, cardiac dysfunction, fever, and severe neutropenia³. Therefore, in clinical practice, dose reductions of cytotoxic anticancer drugs are occasionally required. Despite this, low relative or intended dose intensity (DI) reduces the efficacy of CHOP therapy and maintaining high treatment intensity is important for successful treatment of DLBCL^{6–9}. These DIs may be associated with the pharmacokinetic exposure of cytotoxic anticancer drugs in CHOP therapy^{7,9}. However, the minimum effective and toxic concentrations for each cytotoxic anticancer drug are not clear. In addition, it is unclear whether a dose adjustment based on body surface area (BSA) or age can be used uniformly to establish the pharmacokinetic exposure of these cytotoxic anticancer drugs.

The median age of DLBCL patients in Japan is 70 years old¹, and the number of elderly patients with DLBCL is expected to increase along with the increasing size of the aging population. Elderly patients are generally known to be less tolerant to chemotherapy than adult patients¹⁰. In the last decade, reduced-dose (R-)CHOP regimens, such as (R-)miniCHOP, have been developed^{11–13}. MiniCHOP therapy, which delivers approximate half-doses of the original CHOP regimen, has shown efficacy and safety in patients aged 80 years and older¹¹. Since average global life expectancy has steadily increased, miniCHOP therapy might be useful for elderly patients with DLBCL. Previous studies assessed the treatment intensity of CHOP on the basis of a dose adjustment or an

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	All patients (n = 20)	(R-)CHOP group (n = 12)	(R-)miniCHOP group (n = 8)	P values*
Sex (male:female)	9:11	6:6	3:5	0.465
Age (years)	76 ± 6.6 (67–92)	72 (70–73)	81 (80–85)	–
Body weight (kg)	57.6 ± 11.4 (38.5–77.8)	59.9 (54.8–69.0)	52.1 (42.9–62.3)	0.082
Body surface area (m ²)	1.57 ± 0.18 (1.27–1.91)	1.66 (1.48–1.71)	1.51 (1.32–1.60)	0.181
AST (U/L)	30 ± 14 (15–65)	27 (24–36)	24 (20–39)	0.427
ALT (U/L)	25 ± 17 (10–68)	21 (16–50)	15 (11–22)	0.057
T-Bil (mg/dL)	0.5 ± 0.2 (0.2–0.9)	0.5 (0.4–0.7)	0.5 (0.4–0.6)	0.910
Alb (g/L)	3.3 ± 0.8 (1.8–4.5)	3.7 (3.0–4.3)	2.8 (2.6–3.2)	0.047
eGFR (mL/min)	65 ± 23 (45–131)	70 (53–87)	47 (44–63)	0.020
VCR (mg/body)	1.4 ± 0.3 (1.0–2.0)	1.6 (1.6–1.6)	1.0	–
DXR (mg/body)	53 ± 17 (30–95)	64 (55–65)	35 (30–40)	–
CPA (mg/body)	815 ± 231 (500–1400)	950 (850–1000)	600 (500–650)	–

Table 1. Patient characteristics and administration dose of cytotoxic anticancer drugs. Alb, serum albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPA, cyclophosphamide; DXR, doxorubicin; eGFR, estimated glomerular filtration rate; SD, standard deviation; T-Bil, serum total bilirubin; VCR, vincristine. Values are presented as mean ± SD (range) or median (quartile 1–quartile 3). *(R-)CHOP group vs. (R-)miniCHOP group.

augmented treatment schedule^{6–9}. However, the relationship between dose and blood concentration levels of cytotoxic anticancer drugs in elderly patients receiving CHOP therapy is not sufficiently clear.

In this study, we first investigated the factors that contribute to the effects of individual patient characteristics on the pharmacokinetics of VCR, DXR, and CPA in elderly patients aged 65 years or older. Next, we attempted to establish a simultaneous estimation method for area under the plasma concentration–time curves (AUCs) based on blood concentrations at the same sampling point to monitor the pharmacokinetic exposure of these cytotoxic anticancer drugs in the clinical setting. Finally, we compared the AUCs of VCR, DXR, and CPA between elderly patients aged 80 years and older receiving (R-)miniCHOP therapy and those aged 65–79 years old receiving (R-)CHOP therapy.

Results

Patient characteristics. Patient characteristics are listed in Table 1. A total of 20 patients (9 males and 11 females) were enrolled in this study. Eight patients were 80 years or older and receiving (R-)miniCHOP therapy. None of the patients had severe liver or renal dysfunction. There were no patients taking drugs that obviously affected the pharmacokinetics of VCR, DXR, or CPA, such asazole antifungal agents, cyclosporine, and drug-metabolizing enzyme-inducing antiepileptic drugs^{14–17}. There were significant differences in median serum albumin (Alb) and estimated glomerular filtration rate (eGFR) between (R-)CHOP and (R-)miniCHOP groups (3.7 vs. 2.8 g/L, $P = 0.047$ and 70 vs. 47 mL/min, $P = 0.020$, respectively).

Pharmacokinetic analysis. *Variation of pharmacokinetic parameters.* The pharmacokinetic profiles of each cytotoxic anticancer drug are shown in Fig. 1.

The variations of plasma concentration at each sampling point were larger for VCR and DXR than for CPA. The plasma concentrations of CPA and VCR after 9 and 9.5 h, respectively, from the start of administration generally decreased according to the first-order rate constant; the concentration of DXR showed a biphasic decrease.

Pharmacokinetic parameters of each cytotoxic anticancer drug are listed in Table 2. The coefficients of variation (%CV) of AUC and dose-adjusted AUCs (AUC/Ds) for all cytotoxic anticancer drugs were greater than 15% and those of VCR were the largest of the 3 agents (40.5% and 36.7%, respectively). The %CV of VCR $t_{1/2}$ was larger than that of CPA; the %CV of DXR $t_{1/2}$ could not be calculated because its concentration showed a biphasic decrease. Although the AUC of CPA was significantly correlated with the AUCs of DXR and VCR ($\rho = 0.549$, $P = 0.012$, and $\rho = 0.550$, $P = 0.012$, respectively), there was no significant correlation between the AUCs of DXR and VCR ($\rho = 0.096$, $P = 0.686$).

Relationships between AUC/Ds and patient characteristics. The results of a univariate analysis of variance for AUC/D and $t_{1/2}$ are shown in Table 3. Although there was no significant correlation between any of the patient variables and AUC/D of VCR, body weight and body surface area (BSA) had significant correlations with AUC/D of CPA and DXR ($P < 0.05$). Stepwise multiple linear regression analysis showed that the BSA and Alb were independent factors for predicting the AUC/D of DXR (partial $R^2 = 0.370$, $P = 0.001$, and partial $R^2 = 0.307$, $P = 0.001$, respectively), and BSA was an independent factor for predicting the AUC/D of CPA ($P = 0.002$).

Relationship between AUC and plasma concentrations at each sampling point. The correlations between AUC and plasma concentrations at each sampling point of VCR, DXR, and CPA are shown in Table 4. All plasma concentrations at each sampling point for these cytotoxic anticancer drugs were significantly correlated with AUC (all $P < 0.01$). The correlations between AUC_{1-25} or $AUC_{1.5-25.5}$ and $C_{9 \text{ or } 9.5}$ for each cytotoxic anticancer drug were the

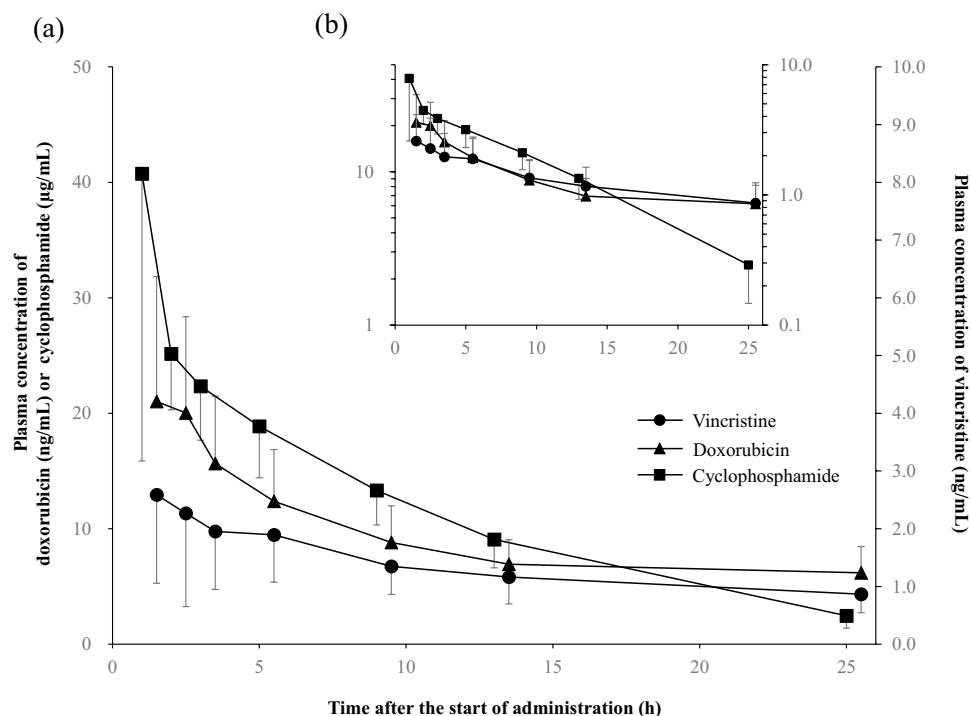


Figure 1. Concentration–time curve of cytotoxic anticancer drugs. **(a)** Linear- and **(b)** logarithmic-scale concentration–time curves of vincristine (circle), doxorubicin (triangle), and cyclophosphamide (square). Each point is presented as a mean \pm standard deviation.

Agents	Parameters	Unit	Mean \pm SD	Median	Range	%CV
Vincristine	$t_{1/2}$	(h)	30.2 \pm 14.1	26.8	(14.0–61.6)	46.7
	$AUC_{1.5-25.5}$	(ng h/mL)	32.0 \pm 12.9	31.6	(11.8–70.9)	40.5
	$AUC_{1.5-25.5}/D$	(ng h/mL/mg)	23.6 \pm 8.7	23.1	(9.9–41.8)	36.7
Doxorubicin	$t_{1/2}$	(h)	Not calculated			
	$AUC_{1.5-25.5}$	(ng h/mL)	219 \pm 69	206	(97–381)	31.6
	$AUC_{1.5-25.5}/D$	(ng h/mL/mg)	4.3 \pm 1.2	4.4	(1.9–6.7)	28.4
Cyclophosphamide	$t_{1/2}$	(h)	6.5 \pm 1.1	6.3	(4.5–8.3)	16.9
	AUC_{1-25}	(µg h/mL)	276 \pm 59	274	(184–386)	21.5
	AUC_{1-25}/D	(µg h/mL/mg)	0.350 \pm 0.069	0.330	(0.270–0.530)	19.6

Table 2. Pharmacokinetic parameters of cytotoxic anticancer drugs in all patients ($n = 20$). $AUC_{n_1-n_2}$, area under the concentration–time curve from n_1 to n_2 h; $AUC_{n_1-n_2}/D$, dose-adjusted area under the concentration–time curve from n_1 to n_2 h; $t_{1/2}$, elimination half-life. %CV, coefficient of variation.

best of all sampling points (all $\rho > 0.9$, $P < 0.001$). The AUC estimation formulas using $C_{9-9.5}$ for each cytotoxic anticancer drug were as follows: $AUC_{1.5-25.5}(\text{VCR}) = 25.16 \cdot C_{9.5} - 2.01$, $R^2 = 0.898$, $P < 0.001$; $AUC_{1.5-25.5}(\text{DXR}) = 20.37 \cdot C_{9.5} + 39.11$, $R^2 = 0.867$, $P < 0.001$; and $AUC_{1-25}(\text{CPA}) = 19.56 \cdot C_9 + 15.55$, $R^2 = 0.972$, $P < 0.001$.

Comparison of AUCs between (R-)miniCHOP and (R-)CHOP. The means \pm standard deviations of ratios of the actual dose to the dose of VCR, DXR, and CPA in the original (R-)CHOP or (R-)miniCHOP regimen were $82\% \pm 6\%$ and $52\% \pm 3\%$, $79\% \pm 7\%$ and $48\% \pm 3\%$, and $79\% \pm 6\%$ and $52\% \pm 2\%$, respectively. Comparisons of the AUC of each cytotoxic anticancer drug between patients receiving (R-)miniCHOP and (R-)CHOP are shown in Fig. 2. The median AUCs of DXR and CPA were significantly lower in the (R-)miniCHOP group than in the (R-)CHOP group [(b) 168.7 vs. 257.9 ng h/mL, $P = 0.003$, and (c) 219.9 vs. 301.7 µg h/mL, $P = 0.020$, respectively]. The median AUC of VCR showed the same trend; however, the difference between the groups was not significant [(a) 24.83 vs. 34.85 ng h/mL, $P = 0.135$].

	Vincristine		Doxorubicin		Cyclophosphamide	
	AUC _{1.5-25.5} /D	t _{1/2}	AUC _{1.5-25.5} /D	t _{1/2}	AUC ₁₋₂₅ /D	t _{1/2}
	Median (ng h/mL/mg or h) of male and female (<i>P</i> values)					
Sex	23.6 vs. 23.1 (0.882)	30.1 vs. 30.4 (0.766)	3.99 vs. 4.75 (0.230)	–	310 vs. 370 (0.016)	6.83 vs. 6.18 (0.175)
	<i>rho</i> (<i>P</i> values)					
Age	0.130 (0.585)	–0.015 (0.950)	0.105 (0.659)	–	0.491 (0.028)	0.210 (0.374)
Body weight	–0.348 (0.132)	–0.121 (0.611)	–0.452 (0.045)	–	–0.465 (0.039)	0.386 (0.093)
BSA	–0.291 (0.213)	–0.120 (0.613)	–0.486 (0.030)	–	–0.587 (0.007)	0.369 (0.110)
AST	–0.078 (0.745)	0.467 (0.038)	0.050 (0.833)	–	0.006 (0.980)	0.198 (0.402)
ALT	0.038 (0.875)	0.050 (0.833)	–0.072 (0.764)	–	–0.259 (0.270)	–0.035 (0.885)
T-Bil	0.061 (0.799)	0.015 (0.950)	–0.326 (0.160)	–	–0.311 (0.182)	0.152 (0.521)
Alb	–0.295 (0.206)	–0.084 (0.723)	–0.690 (0.001)	–	–0.307 (0.187)	0.178 (0.453)
eGFR	–0.247 (0.295)	0.008 (0.972)	–0.274 (0.243)	–	–0.532 (0.016)	–0.116 (0.627)

Table 3. Correlations of dose-adjusted AUC and t_{1/2} with patient variables. Sex (male:female), 9:11. Alb, serum albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC_{n1–n2}/D, dose-adjusted area under the concentration–time curve from n1 to n2 h; BSA, body surface area; eGFR, estimated glomerular filtration rate; T-Bil, serum total bilirubin; t_{1/2}, elimination half-life.

	Vincristine	Doxorubicin	Cyclophosphamide
C ₁ or 1.5	0.945 (<0.001)	0.895 (<0.001)	0.576 (0.008)
C ₂ or 2.5	0.864 (<0.001)	0.933 (<0.001)	0.820 (<0.001)
C ₃ or 3.5	0.872 (<0.001)	0.896 (<0.001)	0.854 (<0.001)
C ₅ or 5.5	0.921 (<0.001)	0.901 (<0.001)	0.861 (<0.001)
C ₉ or 9.5	0.959 (<0.001)	0.951 (<0.001)	0.971 (<0.001)
C ₁₃ or 13.5	0.929 (<0.001)	0.908 (<0.001)	0.915 (<0.001)
C ₂₅ or 25.5	0.882 (<0.001)	0.795 (<0.001)	0.754 (<0.001)

Table 4. Correlations between AUC_{1–25} or 1.5–25.5 and plasma concentration at each sampling point for each cytotoxic anticancer drug. Values are expressed as Spearman's *rho* values (*P* values). AUC_{n1–n2}, area under the concentration–time curve from n1 to n2 h. C_n represents plasma concentration of each anticancer drug at n hours after the start of administration.

Discussion

This study is the first report to show differences in AUCs of VCR, DXR, and CPA between (R-)CHOP and (R-)miniCHOP regimens. In addition, a simple estimation method of AUCs for these cytotoxic anticancer drugs was established. Until now, very few clinical studies have focused on the pharmacokinetics of cytotoxic anticancer drugs in elderly patients receiving CHOP therapy.

The AUCs of DXR and CPA were significantly lower in patients receiving (R-)miniCHOP therapy than in those receiving (R-)CHOP therapy. Although the AUC of VCR was not significantly different between the 2 groups, a similar trend was observed. Because the dose and dose range of VCR were lower and narrower than those of DXR and CPA, there may be no clear differences in these AUCs between groups. At this point, it remains unclear whether elderly patients aged 80 years and older should be treated with equal pharmacokinetic exposures of cytotoxic anticancer drugs as those for patients aged 65–79 years old. Still, it has been reported that the (R-)miniCHOP regimen provides a good balance between efficacy and safety in patients aged 80 years and older with DLBCL^{9,11}. Therefore, the optimal pharmacokinetic exposure of cytotoxic anticancer drugs in these elderly patients may be lower than that of elderly patients aged 65 to 79 years old. Outcomes in elderly patients are worse than in younger patients, but numerous studies have shown that this difference cannot be explained by age alone^{18,19}. Because it is important to complete the 6 cycles in CHOP therapy²⁰, further investigations are required to determine whether the AUCs of cytotoxic anticancer drugs affect the relative DIs.

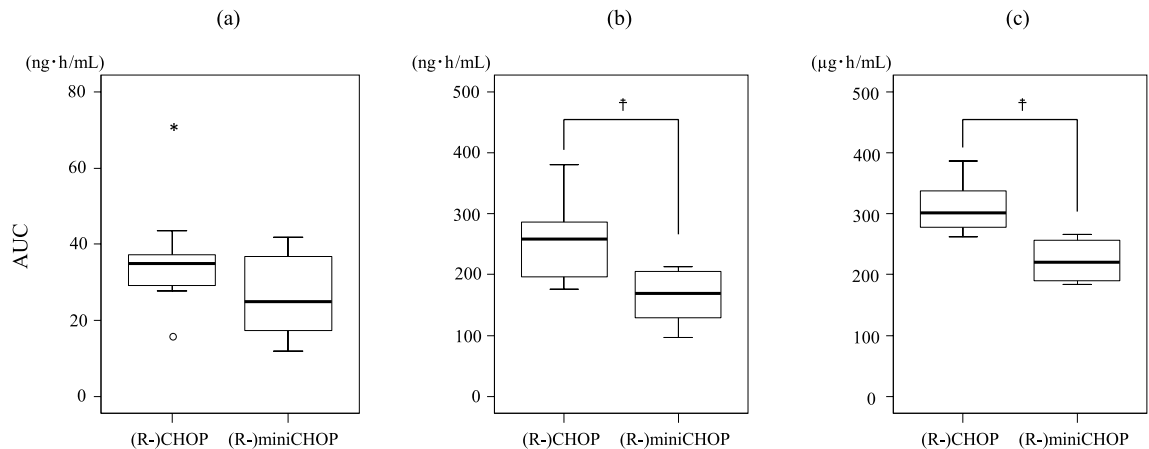


Figure 2. Comparison of AUC of cytotoxic anticancer drugs between (R-)CHOP and (R-)miniCHOP groups. **a** vincristine; **b** doxorubicin; **c** cyclophosphamide. The box spans data between 2 quartiles (interquartile range [IQR]), with the median represented as a bold horizontal line. The ends of the whiskers (vertical lines) represent the smallest and largest values that were not outliers. Outliers (circles) are values between 1.5 and 3 IQRs from the end of the box. Values more than 3 IQRs from the end of the box are defined as extreme (asterisk). AUC, area under the plasma concentration–time curve; AUC of vincristine and doxorubicin, $AUC_{1.5-25.5}$; AUC of cyclophosphamide, AUC_{1-25} ; $^{\dagger}P < 0.05$.

In this study, age was not an independent variable for predicting the AUC/Ds of VCR, DXR, and CPA in elderly patients aged 65 years and older receiving (R-)CHOP or (R-)miniCHOP therapy. Baudry et al. reported that aging did not affect the pharmacokinetics of DXR and CPA in very old patients (≥ 75 years old) receiving R-miniCHOP²¹. Our results agree with the findings of this study. BSA, which was an independent variable for predicting the AUC/Ds of DXR and CPA in this study, has been reported to affect pharmacokinetics parameters in other studies^{22–24}. In addition, in this study, a negative correlation between Alb and AUC/D of DXR was found in elderly patients. It has been reported that DXR clearance (CL) correlates with various liver function test values, including Alb^{25,26}. DXR is eliminated primarily by hepatic metabolism and biliary excretion. Although decreased Alb may represent decreased metabolic capacity, other liver function test values did not affect DXR AUC/D in this study. Further studies are needed to evaluate the relationship between Alb and pharmacokinetic parameters of DXR. Unlike with DXR and CPA, patient factors were not found to impact VCR AUC/D in this study. To the best of our knowledge, the effects of patient factors, such as sex, BSA, and biochemistry covariates, on VCR CL in elderly patients have not yet been clarified. Recently, it has been reported that gene polymorphisms of drug-metabolizing enzymes or transporters affect the pharmacokinetics of these cytotoxic anticancer drugs^{23,27–30}. Thus, various factors have a potential impact on the pharmacokinetic exposure of elderly patients receiving CHOP therapy. However, these covariate factors alone could not fully explain individual variabilities in the pharmacokinetics of VCR, DXR, or CPA. Moreover, the associations between the AUCs of these cytotoxic anticancer drugs and the outcomes of patients with DLBCL are also unclear.

Plasma concentrations at 9–9.5 h after the start of administration were well correlated with AUCs within 24 h from the end of administration in patients receiving VCR, DXR, and CPA. Because accurate measurement of the AUC requires the collection and analysis of multiple blood samples, it is difficult to use this value as an indicator in the clinical setting. On the other hand, the simultaneous estimation method of AUCs of 3 cytotoxic anticancer drugs by a single blood collection at one sampling point is very convenient. The Bayesian estimation method provides a simple technique with good performance for estimating AUC of various drugs. Population pharmacokinetic parameters for DXR^{21,31} and CPA^{21,32} used in the Bayesian estimation method have already been reported. The plasma concentrations at 9–9.5 h after the start of administration are considered the best single sampling point for estimating AUCs of these cytotoxic anticancer drugs according to this Bayesian estimation method. Moreover, estimation of AUCs using a regression line is a simpler analytical method than Bayesian estimation, which requires the use of computer software for analysis. In the future, the AUC estimation formulas for VCR, DXR, and CPA constructed in this study may be useful for examining the relationships between pharmacokinetic exposure and relative DI in CHOP therapy in the clinical setting.

This study had a few limitations. First, the number of patients included in our study population was very small, so associations between AUCs and toxicities, such as severe myelosuppression and peripheral neuropathy, or efficacy, such as progression-free survival or overall survival, were not revealed. Therefore, pharmacokinetics–pharmacodynamics studies consisting of many elderly patients receiving (R-)CHOP or (R-)miniCHOP therapy are needed in the future. Second, the AUCs calculated in this study did not include area under the time during the infusion and the distribution phase, which has a rapid decrease of blood concentration immediately after the end of administration. However, because they cover most of the area of the elimination phase, they can be considered to be indicators of safety and effectiveness. Third, the AUC estimation formulas constructed in this study were derived from a very small number of patients receiving CHOP therapy; thus, validation studies in special populations, such as patients with liver dysfunction, low eGFR, or extreme BSA, are needed. However, our findings revealed that the AUCs of VCR, DXR, and CPA included in CHOP therapy can be simultaneously estimated by

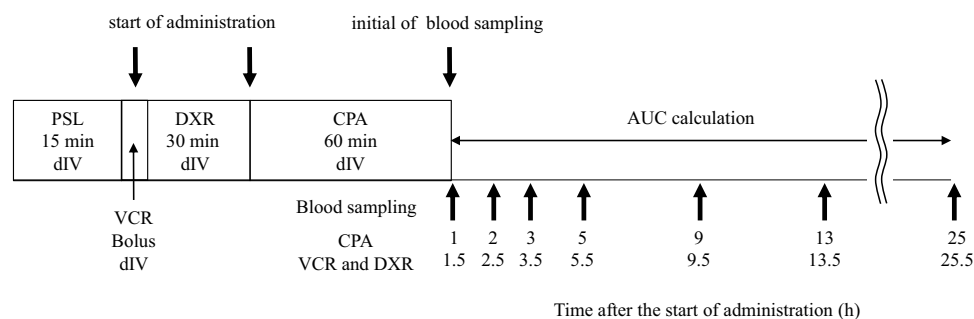


Figure 3. CHOP regimen and blood sampling protocol. AUC, area under the plasma concentration–time curve; CPA, cyclophosphamide; dIV, drip infusion into vein; DXR, doxorubicin; PSL, prednisolone; VCR, vincristine.

a single blood collection at one sampling point in the general elderly population. This AUC estimation method is therefore very simple. In conclusion, it is possible that the AUCs of VCR, DXR, and CPA in patients aged 80 years and older receiving (R-)miniCHOP therapy may be lower than those in patients 65–79 years old receiving (R-)CHOP therapy. However, the relationship between relative DIs and AUCs of cytotoxic anticancer drugs in elderly patients receiving CHOP therapy are not sufficiently clear. Simultaneous estimation of AUCs by a single blood collection at one sampling point may be useful for evaluating the pharmacokinetic exposure of these cytotoxic anticancer drugs in elderly patients receiving (R-)CHOP or (R-)miniCHOP therapy in the future.

Methods

Patients and protocols. This study was carried out at a single institution. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Hiro-saki University Graduate School of Medicine (project identification code: 2018-055). Patients receiving the first round of (R-)CHOP or (R-)miniCHOP therapy at Hiro-saki University Hospital from October 2018 to April 2020 were enrolled in this study. Informed consent was obtained from all individual participants included in this study. The administration regimen for CHOP was as follows: PSL 100 mg intravenous (IV) infusion over 15 min on day 1, VCR 1.4 mg/m² (maximum dose 2.0 mg) IV bolus over 5 min on day 1, DXR 35 mg/m² IV infusion over 30 min on day 1, CPA 750 mg/m² IV infusion over 1 h on day 1, and PSL 100 mg orally on days 2 through 5 (Fig. 3). Rituximab (375 mg/m²) was administered on a different day from CHOP day 1 administration. Approximately 20% dose reductions of VCR, DXR, and CPA were allowed at the discretion of the attending physician in the (R-)CHOP group. Patients who were 80 years and older received (R-)miniCHOP therapy (VCR 1 mg/body, DXR 25 mg/m², CPA 400 mg/m², and PSL 40 mg/m²). Plasma samples were centrifuged at 3500 rpm for 10 min at 4 °C and separated plasma was stored at –80 °C until analysis.

Dose intensity calculation. The DI of each cytotoxic anticancer drug was calculated as a percentage of the dose of the original CHOP regimen. For patients whose BSA exceeded 1.43 m², the DI of VCR was calculated with 2 mg/body as 100%.

Assay of plasma concentrations of VCR, DXR, and CPA. Plasma concentrations of cytotoxic anticancer drugs were measured by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) using the ACQUITY UPLC System (Waters, MA, USA). Pretreatment of plasma samples and UPLC separation were performed based on the method we previously reported³³.

Plasma (100 µL) was mixed with 150 µL of acetonitrile and 10 µL of internal standards (50 ng/mL trofosphamide for DXR and 20 µg/mL cyclophosphamide-d for CPA). The mixture was vortexed for 30 s and centrifuged at 13,500 rpm for 5 min at room temperature. Next, 100 µL of supernatant was diluted with 100 µL of MiliQ for DXR measurement, and 20 µL of supernatant was diluted with 380 µL of MiliQ for CPA measurement. Samples for VCR measurement were prepared according to a different procedure than those for DXR and CPA measurement. Plasma (200 µL) was mixed with 300 µL of acetonitrile and 20 µL of internal standards (50 ng/mL vinblastine [VBL]). The mixture was vortexed for 30 s and centrifuged at 13,500 rpm for 5 min at room temperature. Next, 350 µL of supernatant was evaporated using the centrifuge evaporator and then redissolved with 100 µL of 30% acetonitrile. The mixture was vortexed for 30 s and centrifuged at 13,500 rpm for 1 min at room temperature. The sample was transferred to an autosampler vial and then 2 µL for CPA, 8 µL for DXR, and 10 µL for VCR were injected into an ACQUITY UPLC HSS C18 column (1.7 µm, 100 mm × 2.1 mm) at 40 °C. The mobile phase consisted of (A) MiliQ with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid at a flow rate of 0.4 mL/min. Gradient conditions were as follows: 0–1.0 min, held in 5% B; 1.0–6.0 min, linear from 5 to 95% B; 6.0–7.0 min, held in 95% B; 7.0–7.1 min, linear from 95 to 5% B; 7.1–10.0 min, held in 5% B. The analyte and internal standard were ionized and detected using Xevo TQD (Waters, MA, USA). Positive electrospray ionization was performed in the multiple reaction monitoring mode. Ion transitions were as follows: m/z 413.5 → 353.1 for VCR, 544.2 → 397.0 for DXR, and 265.1 → 140.0 for CPA; internal standards were m/z 811.4 → 751.2 for VBL, 323.0 → 154.0 for trofosphamide, and 265.1 → 140.0 for cyclophosphamide-d. Cone voltage and collision

energies were 60 V and 40 eV for VCR and VBL, 30 V and 20 eV for CPA and cyclophosphamide-d, 30 V and 10 eV for DXR, and 30 V and 30 eV for trofosphamide, respectively. The lower limits of quantitation for VCR, DXR, and CPA were 0.5 ng/mL, 1 ng/mL, and 1 µg/mL, respectively. The calibration curve was linear in the following ranges: VCR, 0.5–10 ng/mL; DXR, 2–80 ng/mL; CPA, 2.5–40 µg/mL. If the plasma concentration of CPA exceeded the upper limit of the standard curve, the sample was diluted by one-third. The intra- and inter-day accuracy values (CV%) were all within $\pm 15\%$ and precision values (CV%) were all less than 15% in each calibration curve range.

Pharmacokinetic analysis. Pharmacokinetic analyses with VCR, DXR, and CPA were carried out using a standard non-compartmental model with Phoenix WinNonlin (Pharsight Co, Mountain View, CA, version 8.1). Plasma samples of CPA, (VCR) and (DXR) were collected at 1 (1.5), 2 (2.5), 3 (3.5), 5 (5.5), 9 (9.5), 13 (13.5) and 25 (25.5) h after the start of each drug administration. The AUCs of each cytotoxic anticancer drug were calculated on the basis of plasma concentrations after initial blood sampling (Fig. 3). Each AUC was calculated using the linear trapezoidal rule. The elimination half-life ($t_{1/2}$) was obtained using the log-linear regression of the terminal phase of the concentration–time data for at least 3 sampling points (elimination half-life = $\ln 2/k_e$; k_e = elimination rate constant).

Statistical procedures. The Shapiro–Wilk test was used to assess distribution. The characteristics of patients and pharmacokinetic parameters of VCR, DXR, and CPA are expressed as means \pm standard deviation or medians and range. The Mann–Whitney U test was used to determine the difference in continuous values between groups. Spearman's rank correlation coefficient test was used to assess correlations in continuous values between groups, and all results are expressed as Spearman's *rho* values. Factors with *P*-values less than 0.05 in a univariate analysis were included in a stepwise multiple linear regression analysis to identify independent factors for predicting results. The estimation formulas were constructed using simple linear regression analysis. The percent variation that could be explained by the simple regression equation was expressed as a coefficient of determination (R^2). A *P* value less than 0.05 was considered statistically significant. Statistical analysis was performed with SPSS 26.0 for Windows (SPSS IBM Japan Inc., Tokyo, Japan).

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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References

- Aoki, R. *et al.* Distribution of malignant lymphoma in Japan: Analysis of 2260 Cases, 2001–2006. *Pathol. Int.* **58**(3), 174–182 (2008).
- Chihara, D. *et al.* Differences in incidence and trends of haematological malignancies in Japan and the United States. *Br. J. Haematol.* **164**(4), 536–545 (2014).
- Coiffier, B. *et al.* CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N. Engl. J. Med.* **346**(4), 235–242 (2002).
- Pfreundschuh, M. *et al.* CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: A randomised controlled trial by the MabThera International Trial (MINT) Group. *Lancet Oncol.* **7**(5), 379–391 (2006).
- Feugier, P. *et al.* Long-term results of the R-CHOP study in the treatment of elderly patients with diffuse large B-cell lymphoma: A study by the Groupe d'Etude Des Lymphomes De l'Adulte. *J. Clin. Oncol.* **23**(18), 4117–4126 (2005).
- Terada, Y. *et al.* Impact of relative dose intensity (RDI) in CHOP combined with rituximab (R-CHOP) on survival in diffuse large B-cell lymphoma. *J. Exp. Clin. Cancer Res.* **28**(1), 116 (2009).
- Hirakawa, T. *et al.* Importance of maintaining the relative dose intensity of CHOP-like regimens combined With rituximab in patients with diffuse large B-cell lymphoma. *Ann. Hematol.* **89**(9), 897–904 (2010).
- Gutiérrez, A. *et al.* Differential impact of relative dose-intensity reductions in diffuse large B-cell lymphoma treated with R-CHOP21 or R-CHOP14. *PLoS ONE* **10**(4), e0123978 (2015).
- Eyre, T. A. *et al.* Impact of intended and relative dose intensity of R-CHOP in a large, consecutive cohort of elderly diffuse large B-cell lymphoma patients treated with curative intent: No difference in cumulative incidence of relapse comparing patients by age. *J. Intern. Med.* **285**(6), 681–692 (2019).
- Peyrade, F., Gastaud, L., Ré, D., Pacquelet-Cheli, S. & Thyss, A. Treatment decisions for elderly patients with haematological malignancies: A dilemma. *Lancet Oncol.* **13**(8), e344–352 (2012).
- Peyrade, F. *et al.* Attenuated immunochemotherapy regimen (R-miniCHOP) in elderly patients older than 80 years with diffuse large B-cell lymphoma: A multicentre, single-arm, phase 2 trial. *Lancet Oncol.* **12**(5), 460–468 (2011).
- Takahashi, A. *et al.* 80% dose R-CHOP is suitable chemotherapy for very elderly patients with DLBCL. *Blood* **126**(23), 5094 (2015).
- Kikuchi, M. *et al.* Reduced-dose (two-thirds) R-CHOP chemotherapy for elderly patients with non-Hodgkin lymphoma. *J. Chemother.* **27**(2), 99–105 (2014).
- Moriyama, B. *et al.* Adverse interactions between azoles and vincristine: review and analysis of cases. *Mycoses* **55**(4), 290–297 (2012).
- Villikka, K., Kivistö, K. T., Mäenpää, H., Joensuu, H. & Neuvonen, P. J. Cytochrome P450-inducing antiepileptics increase the clearance of vincristine in patients with brain tumors. *Clin. Pharmacol. Ther.* **66**(6), 589–593 (1999).
- Rushing, D. A. *et al.* The effects of cyclosporine on the pharmacokinetics of doxorubicin in patients with small cell lung cancer. *Cancer* **74**(3), 834–841 (1994).
- Marr, K. A. *et al.* Cyclophosphamide metabolism is affected by azole antifungals. *Blood* **103**(4), 1557–1559 (2004).
- Hasselblom, S., Ridell, B., Nilsson-Ehle, H. & Andersson, P. O. The impact of gender, age and patient selection on prognosis and outcome in diffuse large B-cell lymphoma—a population-based study. *Leuk. Lymphoma* **48**(4), 736–745 (2007).
- Zinzani, P. L. *et al.* Elderly aggressive-histology non-Hodgkin's lymphoma: First-line VNCOP-B regimen experience on 350 patients. *Blood* **94**(1), 33–38 (1999).

20. Tilly, H. *et al.* Diffuse large B-cell lymphoma (DLBCL): ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* **26**(Suppl 5), v116–v125 (2015).
21. Baudry, E. *et al.* Cyclophosphamide dose adjustment based on body weight and albuminemia in elderly patients treated with R-mini-CHOP. *Cancer Chemother. Pharmacol.* **83**(4), 775–785 (2019).
22. Sandström, M. *et al.* Population analysis of the pharmacokinetics and the haematological toxicity of the fluorouracil–epirubicin–cyclophosphamide regimen in breast cancer patients. *Cancer Chemother. Pharmacol.* **58**(2), 143–156 (2006).
23. Ahmed, J. H. *et al.* Population pharmacokinetic, pharmacogenetic, and pharmacodynamic analysis of cyclophosphamide in Ethiopian breast cancer patients. *Front. Pharmacol.* **11**, 406 (2020).
24. Joerger, M. *et al.* Population pharmacokinetics and pharmacodynamics of doxorubicin and cyclophosphamide in breast cancer patients: A study by the EORTC–PAMM–NDDG. *Clin. Pharmacokinet.* **46**(12), 1051–1068 (2007).
25. Dobbs, N. A. *et al.* Gender affects doxorubicin pharmacokinetics in patients with normal liver biochemistry. *Cancer Chemother. Pharmacol.* **36**(6), 473–476 (1995).
26. Twelves, C. J. *et al.* Doxorubicin pharmacokinetics: the effect of abnormal liver biochemistry tests. *Cancer Chemother. Pharmacol.* **42**(3), 229–234 (1998).
27. Skiles, J. L. *et al.* CYP3A5 genotype and its impact on vincristine pharmacokinetics and development of neuropathy in Kenyan children with cancer. *Pediatr. Blood Cancer* **65**(3), e26854 (2018).
28. Lal, S. *et al.* CBR1 and CBR3 pharmacogenetics and their influence on doxorubicin disposition in Asian breast cancer patients. *Cancer Sci.* **99**(10), 2045–2054 (2008).
29. Lal, S. *et al.* Pharmacogenetics of ABCB5, ABCC5 and RLIP76 and doxorubicin pharmacokinetics in Asian breast cancer patients. *Pharmacogenomics J.* **17**(4), 337–343 (2017).
30. Helsby, N. A. *et al.* The Importance of both CYP2C19 and CYP2B6 germline variations in cyclophosphamide pharmacokinetics and clinical outcomes. *Br. J. Clin. Pharmacol.* **85**(9), 1925–1934 (2019).
31. Pérez-Blanco, J. S. *et al.* Population pharmacokinetics of doxorubicin and doxorubicinol in patients diagnosed with non-Hodgkin's lymphoma. *Br. J. Clin. Pharmacol.* **82**(6), 1517–1527 (2016).
32. Wilde, S. *et al.* Population pharmacokinetics of the BEACOPP polychemotherapy regimen in Hodgkin's lymphoma and its effect on myelotoxicity. *Clin. Pharmacokinet.* **46**(4), 319–333 (2007).
33. Nakagawa, J. *et al.* Impact of gene polymorphisms in drug-metabolizing enzymes and transporters on trough concentrations of rivaroxaban in patients with atrial fibrillation. *Basic Clin. Pharmacol. Toxicol.* <https://doi.org/10.1111/bcpt.13488> (2020).

Author contributions

Conceptualization, T.N.; investigation, T.T., R.H., Y.C., K.H., K.S., K.S., K.H., and K.T.; measurement of anticancer drugs, J.N. and K.U.; formal analysis, J.N.; writing—original draft preparation, J.N.; writing—review and editing, A.S. and T.N. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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