PHARMACOKINETICS

The effects of advanced age and serum α1-acid glycoprotein on docetaxel unbound exposure and dose-limiting toxicity in cancer patients

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AIM

α1-Acid glycoprotein (AAG), which is a major binding protein of docetaxel, is considered to be a determinant for docetaxel pharmacokinetics. However, there are no reports about the impact of serum AAG on pharmacokinetics and pharmacodynamics in elderly patients treated with docetaxel. The aim of this prospective study was to elucidate the effects of advanced age and serum AAG on docetaxel unbound exposure and neutropenia, dose-limiting toxicity, in cancer patients.

METHODS

Docetaxel was administered at 60 mg m^{-2} to 51 patients with non-small cell lung cancer, 17 of whom were ≥75 years of age. Pharmacokinetics, unbound fraction (fu), neutropenia, serum protein levels of AAG and albumin, as well as baseline absolute neutrophil count (ANC) were assessed during the first course. Population pharmacokinetic and pharmacodynamic analyses were performed to evaluate the influence of clinically relevant factors on docetaxel pharmacokinetics and neutropenia.

RESULTS

Clearance of docetaxel and degree of fu were significantly associated with serum AAG level, but not with age. Area under the concentration–time curve of unbound docetaxel (*fu*·AUC) was significantly higher in patients aged ≥75 years (0.389 μg·h ml^{-1}, 95% CI; 0.329–0.448 μg·h ml^{-1}) compared with patients aged <75 years (0.310 μg·h ml^{-1}, 95% CI; 0.268–0.352 μg·h ml^{-1}). Percent decrease in ANC at nadir related to *fu*·AUC, and was dependent on baseline ANC.

CONCLUSION

Regardless of ageing, serum level of AAG determines docetaxel unbound exposure and related dose-limiting toxicity. Serum AAG level and ANC at baseline appear to be predictive of neutropenia for patients of all ages following the administration of docetaxel.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT
- α_1-Acid glycoprotein (AAG), which is a major binding protein of docetaxel, is regarded as a determinant for docetaxel pharmacokinetics.
- Docetaxel-induced severe haematological toxicity occurs more frequently in the elderly, whereas there are no significant age-related differences in the pharmacokinetics.

WHAT THIS STUDY ADDS
- Regardless of ageing, serum AAG level that increases in cancer patients determines docetaxel unbound exposure and related dose-limiting toxicity.
- Neutropenia can be predicted by serum AAG level and absolute neutrophil count at baseline for patients of all ages following the administration of docetaxel.

Introduction

As the population ages, a significant and rapidly increasing number of older cancer patients require treatments appropriate for age-related physiological changes affecting pharmacokinetics and pharmacodynamics in elderly individuals. As such, the International Society of Geriatric Oncology published clinical practice recommendations for dose adjustments of renally excreted cancer drugs in geriatric patients [1, 2] that take into consideration the decline in renal function with ageing [3]. Unfortunately, no dosing information for non-renally excreted cancer drugs has been provided for this population. Predominantly eliminated by hepatic metabolism, docetaxel shows effective antitumor activity against numerous tumours, and is approved for treatment of breast cancer, non-small cell lung cancer (NSCLC), hormone-refractory prostate cancer, gastric adenocarcinoma, and squamous cell carcinoma of the head and neck [4]. Docetaxel monotherapy is one of the standard approaches for pre-treated NSCLC patients [5, 6], and is a first-line treatment for older patients aged ≥70 or 75 years [7–9]. Previous reports suggesting older patients (aged ≥65 or 75 years) were more sensitive to docetaxel-induced neutropenia indicate the pharmacokinetics of docetaxel were unaltered; however, severe haematological toxicity was observed more frequently in the older patient population [10, 11].

As docetaxel is extensively bound to serum proteins (binding percentage > 90%), the concentration of unbound docetaxel correlates with both beneficial and harmful effects of the drug. According to binding parameters, it is thought the major binding protein of docetaxel is α_1-acid glycoprotein (AAG) rather than albumin (ALB) [12]. Hence, variability of serum AAG levels contributes to differences in the unbound fraction and systemic clearance of docetaxel. Area under the concentration–time curve (AUC) of total docetaxel during the first course and baseline serum AAG levels have previously been shown to be independent predictors of treatment efficacy in NSCLC patients [13, 14]. Moreover, first-course total and unbound exposure of docetaxel, as well as baseline serum AAG levels, are related to severity of haematological toxicity [13–16]. Based on these pharmacokinetic and pharmacodynamic characteristics, the aim of this study was to prospectively elucidate the effects of advanced age and serum AAG level on docetaxel unbound exposure and neutropenia, a dose-limiting toxicity, in cancer patients.

Patients and Methods

Eligibility

Patients ≥20 years of age were eligible if they had pathologically confirmed NSCLC with a plan to receive docetaxel monotherapy. Patients with interstitial pneumonitis identified by chest X-ray were excluded.

Study design

The objective was to evaluate the impacts of the advanced age and serum AAG level on unbound docetaxel exposure and neutropenia in cancer patients. Baseline serum levels of AAG and ALB were measured before administration of docetaxel. US National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 was used to assess neutropenia as neutrophil counts decreased [17]. The study duration included the first 3-week course. This study protocol was approved by the institutional review board at Shizuoka Cancer Center in Shizuoka, Japan, where the study was conducted. This study was registered at the University Hospital Medical Information Network Clinical Trials Registry (UMIN 000014701). Study procedures were in accordance with the ethical standards of the Declaration of Helsinki. Written informed consent was obtained from each patient prior to enrolment.

Drug treatment

Docetaxel (Taxotere®; Sanofi K.K., Japan) monotherapy was administered over the course of 1 h at a dose of 60 mg m^{-2} once every 3 weeks to patients with an adequate bone marrow reserve. Dexamethasone was administered at a dose of 8 mg before docetaxel treatment.

Pharmacokinetic sampling and assay

Blood samples were obtained five times: before infusion, just before the end of a 1-h infusion, and at 10–60 min, 2–5 h, and 12–24 h after the end of the infusion. Peripheral blood samples (6 ml) were drawn into vacuumed tubes at each sampling time and centrifuged at 3000 rpm for 10 min at room temperature. Resulting serum was frozen and stored at −80°C until analysis.

Concentration of docetaxel was determined with an ultra-performance liquid chromatography (UPLC)-tandem mass spectrometry technique modified from a previously reported method [18] and developed specifically for this

study. Docetaxel and paclitaxel, an internal standard, were purchased from Sigma-Aldrich (St Louis, MO, USA). The UPLC-tandem mass spectrometry system was equipped with an Acquity UPLC system and a Xevo TQ MS spectrometer (Waters, Milford, MA, USA). Chromatographic separations were obtained under gradient conditions with an Acquity UPLC BEH C18 column (100 mm × 2.1 mm inner diameter, 1.7 μm particle size; Waters). The mobile phase consisted of eluent A (10 mM ammonium formate containing 0.2% formic acid) and eluent B (acetonitrile). The mass spectrometer was run in positive mode, and the multiple reaction monitoring mode detected 808.7 >

Table 1

Patient demographics and characteristics

| Characteristics | Age < 75 years | | | Age ≥ 75 years | | | Total |
	Number of patients	Median	Range	Number of patients	Median	Range	number of patients
Age (years)	34	63	34–73	17	77	75–84	51
BSA (m^2)		1.54	1.37–1.86		1.64	1.19–2.09	
Gender							
Male	28			15			43
Female	6			2			8
AAG (mg dl^{-1})		118	71–264		128	50–249	
ALB (g dl^{-1})		3.7	2.2–4.8		3.7	2.7–4.4	
AST (U l^{-1})		26	14–76		23	15–57	
ALT (U l^{-1})		16	7–57		18	5–40	
Total bilirubin (mg dl^{-1})		0.4	0.2–1.3		0.5	0.2–0.9	
Creatinine, (mg dl^{-1})		0.73	0.42–1.56		0.69	0.44–1.72	
Neutrophil counts per μl		4461	2132–9650		4001	2088–9911	
ECOG performance status							
0	8			2			10
1	25			15			40
2	1			0			1
Prior treatment[a]							
0	1			12			13
1	18			3			21
2	11			2			13
≥3	4			0			4
Smoking status							
Smoker	28			13			41
Never-smoker	6			4			10
Histology							
Adenocarcinoma	28			12			40
Squamous cell carcinoma	5			4			9
Others	1			1			2
EGFR mutation status							
Mutant	7			1			8
Wild	24			12			36
Unknown	3			4			7

AAG, α$_1$-acid glycoprotein; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BSA, body surface area; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor.
[a]$P < 0.05$

226.2 and 854.7 > 105.0 for docetaxel and paclitaxel, respectively. Chromatographic data were acquired and analysed with MassLynx equipped with QuanLynx (Waters).

Concentration of docetaxel in patient serum samples was calculated by determining the ratio of the area of docetaxel to the area of the internal standard in each sample and by comparing these ratios to a standard curve prepared on the same day as the samples. The concentration range of standard curves was 10–4000 ng ml^{-1}. The bias and precision of quality control samples were less than 15%. At the lower limit of assay quantitation, bias and precision were less than 20%, as per guidelines provided in the Food and Drug Administration (FDA) Guidance for Industry Bioanalytic Method Validation [19]. Interday and intraday variabilities in precision (expressed as the coefficient of variation) ranged from 9.2% to 11.8% and from 2.9% to 8.1%, respectively. Average accuracies ranged from 92.3% to 98.3%.

The unbound fraction of docetaxel in serum 10–60 min after the end of infusion was obtained by equilibrium dialysis, which was conducted in a shaking incubator at 37°C for 6 h using 96-well microdialysis plates (HTD96b, HTDialysis, CT, USA). The dialysis compartments in each well were separated by a regenerated cellulose membrane (Dialysis Membrane Strips MWCO 12-14 kDa, HTDialysis). Experiments were carried out with 150-μl plasma aliquots in an equal volume of Dulbecco's Phosphate Buffered Saline (Wako Pure Chemical Industries, Osaka, Japan).

Population pharmacokinetic (PPK) analysis

PPK analysis was performed with NONMEM version 7.3.0 (PDx-POP 4.10; ICON Development Solutions, Dublin, Ireland). The pharmacokinetic model was a two-compartment structural model with first order elimination (subroutines ADVAN3 and TRANS3). Basic pharmacokinetic parameters of docetaxel included total body clearance (CL), volumes of distribution of the central compartment (V_c)
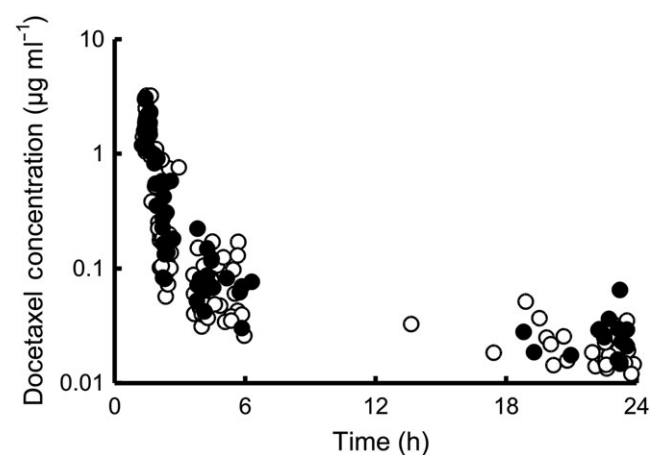
and at steady state (V_{ss}), and intercompartmental clearance (Q). AUC was computed as AUC = Dose/CL.

Interindividual variability was assumed to obey a log-normal distribution and is described for each parameter as follows:

$$\theta j = \theta \times \exp(\eta j) \tag{1}$$

Figure 1

Concentration–time profile of docetaxel in 51 patients. (○) patients aged <75 years, and (●) aged ≥75 years
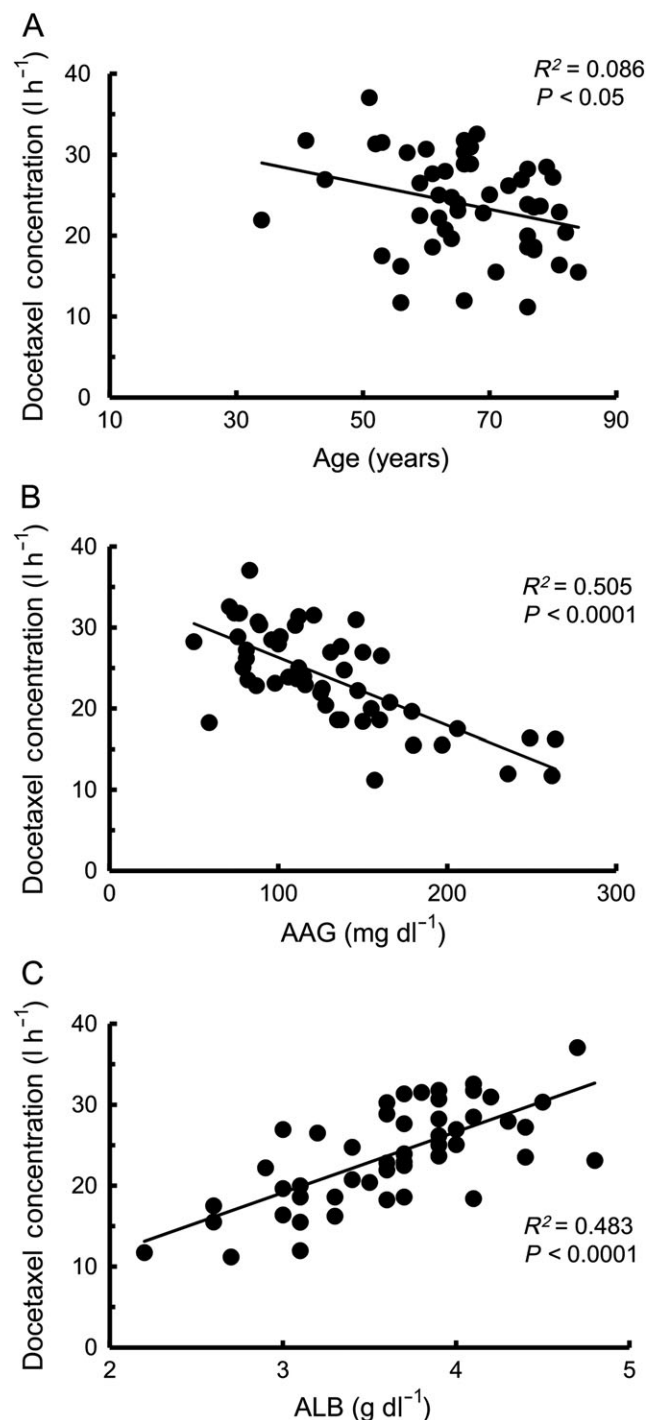
Figure 2

Relationships between total body clearance of docetaxel and age (A), α₁-acid glycoprotein (AAG) level (B), and albumin (ALB) level (C)

where η_j is the random effect for individual j, θ is the population mean parameter, and η is a random variable with mean zero and variance ω^2. Residual variability was described by a proportional error model as follows:

$$C_{i,j} = \text{Cpred}_{i,j} \exp\left(\varepsilon_{i,j}\right) \qquad (2)$$

where $\text{Cpred}_{i,j}$ is the ith model-predicted concentration for patient j, $C_{i,j}$ is the measured concentration, and $\varepsilon_{i,j}$ denotes the residual intraindividual random error.

Demographic variables of age, as well as serum levels of AAG and ALB, were examined to identify whether these variables could explain the observed substantial interindividual variability. Demographic variables, which were considered continuous, were included one at a time by stepwise selection based on a likelihood ratio test. Minimum values of the NONMEM objective function were used as a statistic for choosing suitable models during the model-building process. Potentially significant covariates were identified as factors that, when added to the basic model individually, resulted in a decrease in the objective function of 3.84 or more ($P < 0.05$).

Exposure-toxicity analysis

The percent decrease in absolute neutrophil count (ANC) was defined as:

$$\text{Percent decrease in ANC} = \frac{\text{Pretreatment ANC} - \text{Nadir ANC}}{\text{Pretreatment ANC}} \times 100 \qquad (3)$$

and the relationship between percent decrease in ANC at nadir and unbound AUC (*fu*·AUC) of docetaxel was described by a sigmoid $E_{\max}$ model:

$$\text{Percent decrease in ANC} = \frac{\text{Emax} \times [fu \cdot \text{AUC}]^\gamma}{\text{EC}_{50}{}^\gamma + [fu \cdot \text{AUC}]^\gamma} \qquad (4)$$

where $E_{\max}$ represents the maximal effect and EC_{50} is the *fu*·AUC value at which 50% of the maximum effect occurs. The exponent γ is a shape factor that determines the steepness of the response curve. Covariates capable of affecting $E_{\max}$ and EC_{50} were tested for age, gender and baseline ANC by univariate and multivariate analyses. Computations were made using NONMEM.

Table 2

Final estimates of population pharmacokinetics and exposure-toxicity parameters of total docetaxel

Parameters	Estimate	Standard error of estimate	95% Confidence interval
Population pharmacokinetics			
CL (l h^{-1}) = θ_1*BSA*(AAG/121)$^{\theta 2}$			
θ_1	14.5	0.516	13.5, 15.5
θ_2	−0.495	0.0816	−0.655, −0.335
V$_c$ (l) = θ_3*(ALB/3.7)$^{\theta 4}$			
θ_3	8.89	0.403	8.10, 9.68
θ_4	−0.550	0.185	−0.913, −0.187
V$_{ss}$ (l)	129	8.28	113, 145
Q (l h^{-1})	11.0	0.542	9.94, 12.1
Interindividual variability			
ω^2_{CL}	0.0426	0.00903	0.0249, 0.0603
ω^2_{Vc}	0.00885	0.00923	0, 0.0269
ω^2_{Vss}	0.0176	0.00987	0, 0.0369
Intraindividual variability			
σ^2	0.0360	0.00598	0.0243, 0.0477
Exposure-toxicity relationship for neutropenia			
E$_{max}$ (%)	84.5	2.13	80.3, 88.7
EC$_{50}$ (µg·h ml^{-1}): Baseline ANC > 4341	0.142	0.00267	0.137, 0.147
EC$_{50}$ (µg·h ml^{-1}): Baseline ANC ≤ 4341	0.101	0.0159	0.0698, 0.132
Shape parameter, γ	3.72	0.518	2.70, 4.74
Residual variability	0.0164	0.00531	0.00599, 0.0268

AAG, α_1-acid glycoprotein level; ALB, albumin level; ANC, absolute neutrophil count; BSA, body surface area; CL, total body clearance; E$_{max}$, maximum effect on percent decrease in absolute neutrophil counts; EC$_{50}$, *fu*·AUC value that causes 50% of the maximum effect; Q, inter-compartmental clearance; V$_c$, volume of distribution of the central compartment; V$_{ss}$, volume of distribution at steady state; ω^2, variance of interindividual variability of parameters; σ^2, variance of intraindividual variability.

Statistical analysis

All categorical variables were analysed using a chi-square or Fisher's exact test, as appropriate. Continuous variables between two groups were analysed using the Mann–Whitney U-test. Linear regression analysis was used to evaluate the association between continuous variables and docetaxel pharmacokinetic parameters. All P-values were reported as two-sided and values < 0.05 were considered statistically significant.

Nomenclature of targets and ligands

Key ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [20].

Results

Between August 2014 and May 2015, 51 Japanese patients (34 aged <75 and 17 aged ≥75 years) were enrolled into this study. All patients were assessable for pharmacokinetics, and 49 patients were also evaluated for neutropenia because two patients were excluded due to prophylactic administration of granulocyte colony-stimulating factor (CSF). Patient demographics and characteristics listed in Table 1 were similar between patients aged <75 years and patients aged ≥75 years, except for the number of prior treatments. Older patients had received fewer previous chemotherapies ($P < 0.0001$). No significant differences in serum levels of AAG and ALB, or ANC at baseline existed between the two cohorts.

Pharmacokinetics

Serum concentration–time profiles of total docetaxel in patients aged ≥75 years and <75 years are shown in Figure 1. Individual CL was plotted against a patient's factors to examine any correlations (Figure 2). A moderate association was observed between decreased CL and increased age ($R^2 = 0.086$, $P < 0.05$; Figure 2A), whereas strong correlations existed between CL and serum AAG level ($R^2 = 0.505$, $P < 0.0001$; Figure 2B) and inversely between CL and serum ALB level ($R^2 = 0.483$, $P < 0.0001$; Figure 2C). According to univariate analyses, CL, V_c and V_{ss} of docetaxel were significantly associated with serum levels of both AAG and ALB ($P < 0.001$), but not with age (Table S1). Multivariate analysis showed that serum AAG level was the only significant factor affecting CL of docetaxel ($P < 0.001$; Table S1). However, serum ALB level was significantly associated with V_c ($P < 0.005$; Table S1). Estimated PPK parameters are summarized in Table 2.

Pharmacokinetic parameters in patients aged <75 years and ≥75 years of age are listed in Table 3. Total docetaxel pharmacokinetic parameters, including C_{max}, V_c and V_{ss}, were not different between the two cohorts. CL per individual (l h^{-1}) was significantly higher in patients aged <75 years ($P < 0.05$); however, CL per body surface area (l h^{-1} m^{-2}) was not significantly different between the two cohorts. Total docetaxel AUC and degree of unbound fraction (fu) were higher in patients aged ≥75 years (4.49 ± 1.19 μg·h ml^{-1} and

Table 3

Pharmacokinetic parameters and incidence of neutropenia

	Age < 75 years	Age ≥ 75 years
Pharmacokinetic parameters	n = 34	n = 17
Total docetaxel		
C_{max} (μg ml^{-1})	1.76 ± 0.60	2.17 ± 1.18
AUC (μg·h ml^{-1})	4.09 ± 1.28	4.49 ± 1.19
CL (l h^{-1})	25.12 ± 5.98	21.49 ± 4.84*
CL (l h m^{-2})	15.65 ± 3.92	13.87 ± 3.30
V_c (l)	9.05 ± 1.00	9.19 ± 0.86
V_c (l m^{-2})	5.66 ± 0.87	5.97 ± 0.87
V_{ss} (l)	129.47 ± 9.09	126.37 ± 9.29
V_{ss} (l m^{-2})	81.06 ± 11.90	81.81 ± 9.76
Unbound docetaxel		
Unbound fraction (fu)	7.8 ± 2.7%	8.9 ± 2.1%
fu·AUC (μg·h ml^{-1})	0.310 ± 0.121	0.389 ± 0.114*
Neutropenia	n = 33	n = 16
Percent decrease in ANC at nadir	76.4 ± 13.2	85.2 ± 9.3*
No. of patients		
Grade 4	7 (22%)	8 (50%)#
Grade 3	9 (27%)	4 (25%)
Grade 2	9 (27%)	2 (13%)
Grade 1	4 (12%)	0
Grade 0	4 (12%)	2 (13%)

Data are shown as the mean ± standard deviation.
ANC, absolute neutrophil count; AUC, area under the concentration–time curve; CL, total body clearance; C_{max}, maximum plasma concentration; V_c, volume of distribution of the central compartment; V_{ss}, volume of distribution at steady state.
*Statistically significant difference at $P < 0.05$ using the Mann–Whitney U test.
#$P < 0.05$ compared between the two cohorts with or without incidence of grade 4 neutropenia as assessed using the Fisher's exact test.

8.9 ± 2.1%, respectively) than in patients aged <75 years (4.09 ± 1.28 μg·h ml^{-1} and 7.8 ± 2.7%, respectively). However, this observation did not reach a level of statistical significance ($P = 0.084$ and $P = 0.071$, respectively). But fu·AUC, which was calculated as the product of total AUC and unbound fraction, was significantly higher in older patients (0.389 ± 0.114 μg·h ml^{-1}) than in other patients (0.310 ± 0.121 μg·h ml^{-1}, $P < 0.05$).

Unbound fraction

Degree of unbound fraction varied from 3.1% to 15.6% (median = 8.1%) in 51 patients, as shown in Figure 3A. There was no association between degree of unbound fraction and age ($R^2 = 0.042$; Figure 3B), in accordance with no significant
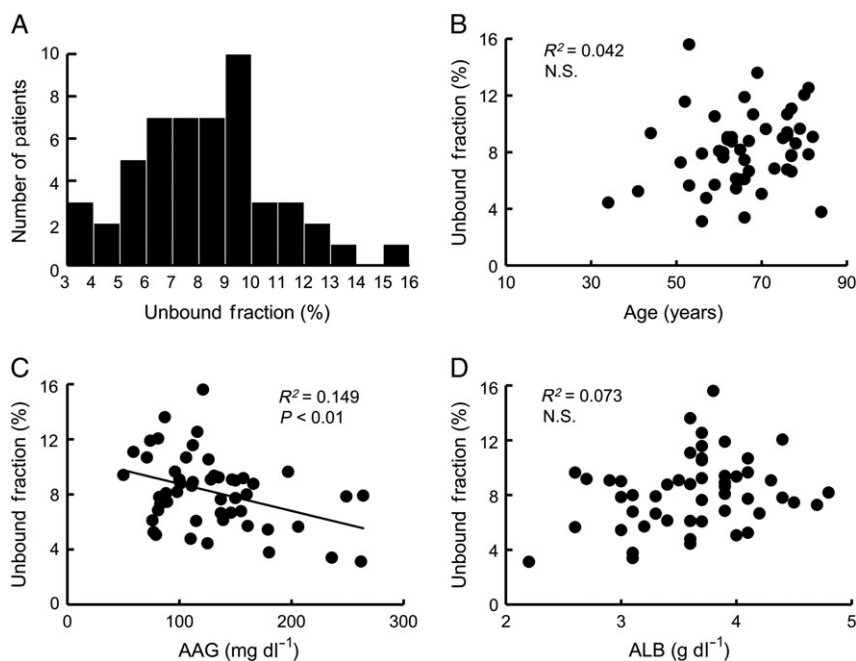
Figure 3

Distribution of the unbound fraction of docetaxel (A) and correlations between the degree of unbound fraction and age (B), α_1-acid glycoprotein (AAG) (C) and albumin (ALB) (D). N.S., not statistically significant

difference in the degree of unbound fraction between patients aged <75 years and ≥75 years, as shown in Table 3. The degree of unbound fraction weakly correlated with serum AAG levels ($R^2 = 0.149$, $P < 0.01$; Figure 3C), but not with serum ALB ($R^2 = 0.073$; Figure 3D).

Neutropenia

Thirty-three patients aged <75 years and 16 patients aged ≥75 years were assessable for neutropenia. Only one patient experienced febrile neutropenia. Percent decrease in ANC at nadir and the number of patients with each grade of neutropenia are summarized in Table 3. Percent decrease in ANC at nadir was significantly greater in patients aged ≥75 years (85.2 ± 9.3%) than in patients aged <75 years (76.4 ± 13.2%, $P < 0.05$). As shown in Figure 4A, percent decrease in ANC at nadir was related to $fu\cdot$AUC, but not to total AUC ($P = 0.628$, not shown). Exposure-toxicity analysis using Eq. (4) revealed that EC_{50} was dependent on ANC value at baseline, but not on age and gender (Table S2, Table 2 and Figure 4A). In addition, the percentage of patients with grade 4 neutropenia was higher in the ≥75 years group ($P < 0.05$; Table 3). The box plot in Figure 4B shows a decrease in ANC at baseline occurs with increasing grade of neutropenia ($P < 0.05$).

Discussion

Docetaxel pharmacokinetic and pharmacodynamic profiles in elderly individuals are still unclear because of large interpatient variability caused by physiological and pathophysiological individual changes. The serum level of AAG, a major binding protein of docetaxel, is considered to be a determinant for docetaxel pharmacokinetics and pharmacodynamics. However, there are no reports about the impact of serum AAG on exposure and toxicity in elderly patients treated with docetaxel. Therefore, this prospective study investigated the effects of advanced age and serum AAG level on pharmacokinetics and related neutropenia of docetaxel in cancer patients by assessing actual unbound drug fraction (fu). Docetaxel CL was found to be associated with serum AAG level regardless of age (Figure 2, Table S1 and Table 2). AAG is an acute phase reactant, and its serum level is increased in disease states such as cancer, burns and acute myocardial infarction [21]. It is also known that serum ALB levels are low in critically ill patients because of altered distribution between intravascular and extravascular compartments [22]. Thus, decreased ALB levels might mitigate increases in AAG, explaining why no effect is observed in some of the studies that focus on AAG only. Therefore, the correlation between CL and serum ALB level, observed to be significant in univariate analysis (Figure 2, Table S1), is deemed to be solely apparent. ALB is a significant covariate affecting V_c but not CL (Table S1, Table 2). In a previous study, AAG was identified to be a significant predictor of total docetaxel CL, with high AAG levels being associated with reduced total docetaxel CL [23]. Our result is consistent with this report and verifies this relationship in a wide range of ages from 34 to 84 years. This observation also shows that docetaxel has a low hepatic extraction ratio because the unbound fraction, as determined by AAG level (Figure 3C), correlated with total CL. In addition, moderate hepatic dysfunction has been reported to decrease docetaxel CL [23–25]. However, only one patient aged <75 years had moderate hepatic dysfunction (AST 76 U l^{-1}, ALT 57 U l^{-1}) in our study, as shown in Table 1.
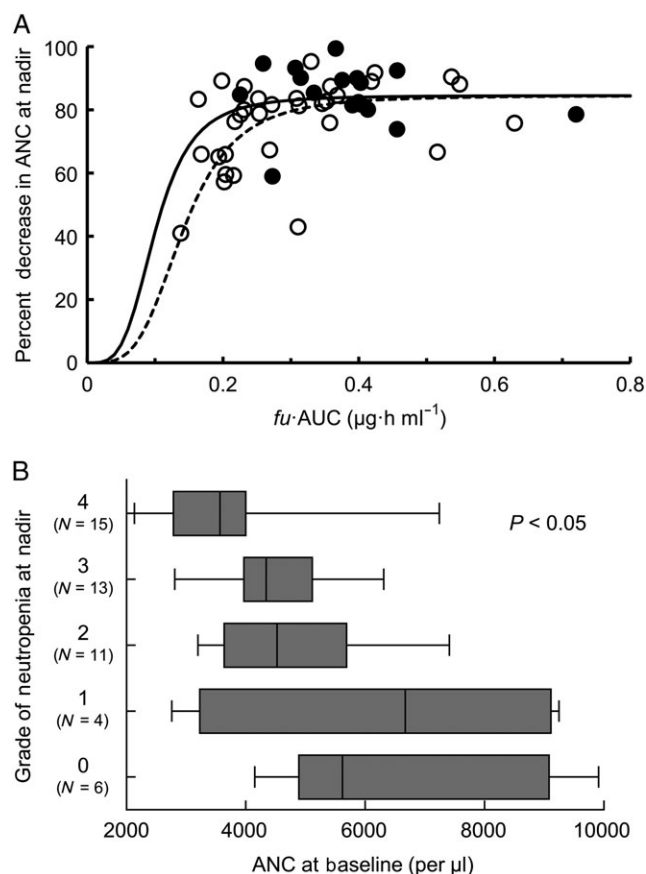
Figure 4

Associations between percent decrease in absolute neutrophil count (ANC) at nadir and unbound exposure ($fu \cdot AUC$) in patients aged <75 years (○) and patients aged ≥75 years (●) (A); Solid and dashed lines represent prediction by a sigmoid E_{max} model in patients with baseline ANC ≤ 4341 μl^{-1} and > 4341 μl^{-1}, respectively. Box plot of neutropenia grade vs. ANC at baseline (B); Box extending from the 25th to 75th percentile with the 50th percentile drawn inside the box and a line extending to the 95th percentile

Therefore, the influence of hepatic function on docetaxel pharmacokinetics was not evaluable in this patient population. Other currently known factors of interindividual variability in docetaxel pharmacokinetics are not believed to have major impact [26].

Generally, older NSCLC patients who received chemotherapy had fewer previous chemotherapy events than younger patients and were less likely to receive platinum-based regimens. Nevertheless, older patients (aged ≥75 years) had more adverse events during chemotherapy, independent of comorbidity [27]. This current study of NSCLC patients shows no age-related differences in total docetaxel pharmacokinetic parameters, but higher incidence of grade 4 neutropenia in patients aged ≥75 years (Table 3) despite the number of prior treatments being fewer in older patients (Table 1). These results are consistent with other studies in various cancer patients treated with docetaxel [10, 11]. Ten Tije et al. [10] and Hurria et al. [11] reported no statistically significant age-related differences in total docetaxel pharmacokinetics; however, older patients (aged ≥65 or 75 years) experienced severe

haematological toxicity at doses of 75 mg m^{-2} once every 3 weeks or 35 mg m^{-2} weekly, respectively. Dosage of docetaxel in our study was 60 mg m^{-2} once every 3 weeks, which differed from the recommended dose of 75 mg m^{-2} for NSCLC in the US and Europe. There is still discrepancy in clinically feasible standard doses for NSCLC between Japan and other countries, as docetaxel was approved in Japan at a recommended dose of 60 mg m^{-2} once every 3 weeks based on the results of Japanese registration studies [28–30]. There are no racial and/or ethnic differences in docetaxel pharmacokinetics, but a marked difference has been observed in toxicities of docetaxel [31].

Unbound drugs are pharmacologically active because they can traverse cell membranes and distribute into tissues to bind their targets. It has been reported that exposure of unbound docetaxel is related to drug-induced hematologic toxicity [15, 16]. We illustrated that percent decrease in ANC at nadir was related to $fu \cdot AUC$ (Figure 4A). Interestingly, our results indicate unbound AUC ($fu \cdot AUC$) of docetaxel was significantly higher in patients aged ≥75 years than in patients aged <75 years (Table 3), suggesting severe neutropenia in older patients is attributable not only to high sensitivity as concluded in previous reports, but also high unbound AUC ($fu \cdot AUC$). As serum AAG level is a determinant of CL (Table S1, Table 2), which inversely relates to AUC, as well as unbound fraction (fu; Figure 3C), AAG levels determine $fu \cdot AUC$ and can predict docetaxel-induced neutropenia for even older patients, with a wide degree of interindividual variability.

Low baseline ANC patients are reportedly at an increased risk of developing grade 4 neutropenia. We also found ANC at baseline to be a significant factor affecting EC_{50}, which in turn influenced the percent decrease in ANC induced by docetaxel (Table S2, Table 2 and Figure 4A). Further, decreased ANC at baseline correlated with development of severe neutropenia (Figure 4B). Indeed, in patients aged ≥75 years whose baseline ANC tended to be lower but not significantly compared with patients aged <75 years (Table 1), the percentage of patients with grade 4 neutropenia was significantly higher (Table 3). Therefore, low ANC at baseline can predict severe neutropenia as it is described in the clinical practice guideline "Recommendations for the Use of WBC Growth Factors" by the American Society of Clinical Oncology (ASCO) that pre-existing neutropenia is one of the risk factors for febrile neutropenia [32].

The ASCO guideline also recommends primary prophylaxis with CSF for patients with risk factors of febrile neutropenia including those aged ≥65 years. Increasing risk and severity of toxicities with age can be explained in terms of declining bone marrow reserve [33], dysregulated cellular proliferation, decreased ability to repair DNA, and reduced immune surveillance. Serum ALB levels are generally decreased in the elderly, whereas AAG levels are not altered by age per se [34]. However, in cancer patients, serum AAG levels have been reported to be high and vary approximately five-fold between individuals [35]. Indeed, serum AAG levels at baseline varied from 50 to 264 mg dl^{-1} (normal range; 39–98 mg dl^{-1}), and consequent degree of unbound fraction was also widely distributed in 3.1–15.6% (Figure 3A).

In conclusion, this study indicates that serum level of AAG is a key determinant of docetaxel unbound exposure

and related dose-limiting toxicity regardless of ageing. Neutropenia can be predicted by serum AAG level and ANC at baseline for patients of all ages following the administration of docetaxel.

Competing Interests

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Supporting Information

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Table S1 Covariate search for factors affecting pharmacokinetics of total docetaxel
Table S2 Covariate search for factors affecting exposure-toxicity relationship of docetaxel (percent decrease in ANC at nadir)