Contribution of Plasma Proteins, Albumin and Alpha 1-Acid Glycoprotein, to Pharmacokinetics of a Multi-targeted Receptor Tyrosine Kinase Inhibitor, Sunitinib, in Analbuminemic Rats

YUKIO TOYAMA[1,4], JUN UEYAMA[2], HIROSHI NOMURA[2], IKUTO TSUKIYAMA[1], HIROKO SAITO[3], TATSUYA HISADA[4], KATSUHIKO MATSUURA[1] and TAKAAKI HASEGAWA[1]

[1]*Department of Hospital Pharmacy, Aichi Medical University School of Medicine, Nagakute, Aichi, Japan;*
[2]*Department of Pathophysiological Laboratory Sciences, Nagoya University Graduate School of Medicine, Higashi-ku, Nagoya, Aichi, Japan;*
[3]*Faculty of Pharmacy, Meijo University, Tenpaku-ku, Nagoya, Aichi, Japan;*
[4]*Department of Pharmacy, Toyota Memorial Hospital, Toyota, Aichi, Japan*

Abstract. *The present study investigated the role of the major plasma proteins, albumin and α_1-acid glycoprotein (AAG), in the pharmacokinetics of sunitinib using Sprague-Dawley (SD) rats and analbuminemic rats with considerably low concentration of albumin established from SD rats. When sunitinib (3 mg/kg) was administered intravenously, the plasma concentrations of sunitinib at the early-distribution phase were significantly lower in analbuminemic rats than those in SD rats. The corresponding pharmacokinetic parameters of systemic clearance and volume of distribution at steady-state of sunitinib were significantly larger in analbuminemic rats (2.17 l/h/kg and 3.94 l/kg, respectively) than those in SD rats (1.26 l/h/kg and 2.37 l/kg, respectively). In in vitro protein-binding experiments using an equilibrium dialysis method, the binding profiles of sunitinib in SD and analbuminemic rats were linear, and the unbound fraction in analbuminemic rats (0.110) was significantly larger than that of SD rats (0.062). However, no significant differences in the unbound plasma concentration–time curves and pharmacokinetic parameters of sunitinib were observed between SD and analbuminemic rats. Protein-binding profiles of sunitinib to human serum albumin and AAG showed concentration independency and the binding potency was 65.3% and 33.7%, respectively. These results suggest that AAG has a low affinity for sunitinib and that the contribution of AAG to plasma protein-binding of sunitinib is relatively low compared to albumin. The present study suggests that the increased systemic clearance of sunitinib in analbuminemic rats might be due to an increase in the volume of distribution at steady-state, which could be due to the significant increase in the unbound fraction of sunitinib due to the low concentration of albumin.*

Correspondence to: Takaaki Hasegawa, Department of Hospital Pharmacy, Aichi Medical University School of Medicine, Karimata, Yazako, Nagakute, Aichi 480-1195, Japan. Tel: +81 561623311, Fax: +81 561625293, e-mail: takahase@aichi-med-u.ac.jp

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Sunitinib, a multi-targeted receptor tyrosine kinase inhibitor, inhibits vascular endothelial growth factor receptors 1-3, which play key roles in angiogenesis and vasculogenesis, platelet-derived growth factor receptors (α and β), stem cell factor receptor (KIT), fms-like tyrosyl kinase-3, and colony-stimulating factor-1 receptor (1, 2). Based upon such inhibitory actions, sunitinib is used for the treatment of advanced and metastatic renal cell carcinoma and imatinib-resistant gastrointestinal stromal tumors.

It is well-known that drug permeability through biological membranes depends on factors such as plasma protein-binding, hydrophobicity and the molecular size of the drug. Among these factors, protein-binding of the drug, which is closely-related to its hydrophobicity, plays an important role in drug pharmacokinetics and pharmacodynamics.

Sunitinib is a basic drug and has physicochemical properties such as high hydrophobicity and high plasma protein-binding potency (3). For example, the log of the solubility of sunitinib in octanol to that in water, which represents hydrophobicity, is extremely high at 5.2 (2). Based on these characteristics, it is considered that sunitinib has a large volume of distribution (V_{SS}) because it can easily pass through various types of tissue membrane. On the other hand, it is generally accepted that basic drugs mainly bind to α_1-acid

glycoprotein (AAG), as well as to albumin and lipoproteins. Therefore, the pharmacokinetics of sunitinib might be altered in patients with cancer since plasma concentration of AAG are elevated in patients with inflammation or diseases, including cancer (4, 5), whereas albumin decreases due to malnutrition and inflammation (6, 7).

There are interesting clinical reports suggesting that there is a significant linear relationship between the plasma concentration of AAG and imatinib (8, 9) which has almost the same degree of protein-binding as sunitinib (10). These clinical findings suggest that AAG plays a more important role than albumin and lipoproteins in the pharmacokinetics of imatinib. It is, therefore, possible that changes in the AAG concentration and the ratio of AAG to albumin alter the pharmacokinetics of sunitinib in patients with cancer. To our knowledge, little information on the role of either albumin or AAG in the pharmacokinetics of sunitinib is available.

Nagase analbuminemic rats, established from Sprague-Dawley (SD) rats, are characterized by a considerably low plasma albumin concentration and hyperlipidemia (11). A number of studies on the pharmacokinetic characteristics of various drugs in analbuminemic rats have been reported (12-16). We have also reported that there are no significant differences in the pharmacokinetics of the anti-fungal antibiotic micafungin and expression of the hepatic ATP-binding cassette C2 protein (Abcc2) between SD and analbuminemic rats (17). Our studies demonstrated that other plasma proteins rather than albumin contribute to the pharmacokinetics of micafungin in analbuminemic rats.

The aim of the present study was to establish a guideline for the safe use of sunitinib for patients who are scheduled to receive sunitinib therapy, and to clarify the role of plasma proteins such as AAG and albumin in the pharmacokinetics of sunitinib in these patients. To investigate whether there are differences in the pharmacokinetics of sunitinib between SD and analbuminemic rats, sunitinib was administered intravenously. We also investigated its plasma protein-binding characteristics to albumin and AAG *in vitro* using an equilibrium dialysis method.

Materials and Methods

Chemicals. Sunitinib malate was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Imatinib mesilate, which was used as an internal standard for the measurement of sunitinib concentrations in plasma and in samples obtained from the *in vitro* protein-binding experiments, was purchased from BioVision (Milpitas, CA, USA). Sekijuji albumin 5% *i.v.* (12.5 g/250 ml), which was purchased from Japanese Red Cross Society (Tokyo, Japan), was used as human serum albumin (HSA). Human α_1-acid glycoprotein (AAG) was purchased from Sigma-Aldrich, Co. (St. Louis, MO, USA). All other chemicals were commercially-available and were of the highest purity available. All materials were used without further purification. For animal experiments, sunitinib was dissolved in citric acid buffer (pH 4.7) at a concentration of 3 mg/ml.

Animals and experiments. Male SD rats (age: 9 weeks; body weight: 342 to 360 g) and male Nagase analbuminemic rats (body weight: 340 to 365 g) of the same age as SD rats were obtained from Japan SLC (Hamamatsu, Japan). The rats were housed under controlled environmental conditions (temperature of 23±1˚C and humidity of 55±5%) with a commercial diet and water freely-available to animals. All animal experiments were carried out in accordance with the guidelines of Nagoya University for the care and use of laboratory animals (024-022).

One day before examination, rats under anesthesia by intraperitoneal injection of sodium pentobarbital (25 mg/kg of body weight) were cannulated with polyethylene tubes into the right jugular vein for drug administration and blood sampling. The rats received a bolus injection of sunitinib (3 mg/kg). Control rats received a bolus injection of saline (0.1 ml/100 g). Blood samples (<0.2 ml) were collected at designated time intervals (5, 10, 20, 30, 45 min and 1, 2, 4, 6, 8, and 10 h after injection of sunitinib). Plasma samples were obtained from the blood samples by centrifugation at 3,000 ×*g* for 10 min at 4˚C and stored at −70˚C until analysis.

In vitro protein-binding study. The plasma protein-binding of sunitinib was determined by equilibrium dialysis using a cellulose membrane (Visking sheet; Sanplatec Corp., Osaka, Japan) with molecular cut-off of 10,000 to 20,000 according to our previous studies (18). Blood samples (approximately 7 ml) were obtained from SD and analbuminemic rats (n=3, respectively) by exsanguination from the abdominal aorta under light ether anesthesia, and plasma samples were obtained by centrifugation at 3,000 ×*g* for 5 min. Each obtained plasma sample (approximately 3 ml) was mixed to determine the binding profiles of sunitinib. Sunitinib-spiked plasma samples of desired concentrations were immediately dialyzed against an equal volume (0.5 ml) of saline at 37˚C for 20 h. The time required to reach equilibrium was determined beforehand. After dialysis, concentrations of sunitinib on both sides of the membrane were measured.

To evaluate the protein-binding characteristics of sunitinib to HSA and AAG, an aliquot (0.5 ml) of sunitinib-spiked 4.5% HSA or 0.1% AAG solution with the appropriate concentration range for sunitinib was dialyzed against an equal volume of saline according to the method described above. The HSA and AAG solution was prepared with saline. Concentrations of HSA and AAG were set using the standard reference value for humans.

Assay methods. Concentrations of sunitinib in each sample *in vitro* and *in vivo* were determined by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS 6430 detection system; Agilent Technologies, Santa Clara, CA, USA) according to a reported method (19) with minor modification (20). Sample preparation procedures differed between plasma and other samples obtained from *in vitro* protein-binding experiments. Sample mixtures containing 20 μl of plasma, 380 μl of distilled water, 800 μl of methanol and 50 μl of the internal standard solution of imatinib (10 μg/ml) were passed through a solid-phase extraction column, Captiva ND^{ripid} (Agilent Technologies), for removing proteins and lipids. The eluent was applied to LC-MS/MS analysis. On the other hand, samples obtained from *in vitro* protein-binding experiments such as 4.5% HSA, 0.1% AAG and saline were analyzed after de-proteination by acetonitrile. Briefly, 10 μl of each sample, 490 μl of distilled water, 500 μl of acetonitrile and 10 μl of internal standard solution were mixed. After centrifugation (12,000 ×*g* for 5 min at 4˚C), the supernatant was injected into LC-MS/MS.

Table I. *Biochemical data for Sprague-Dawley (SD) rats and analbuminemic rats.*

Animal	Concentration (mean±SD [n=4])				
	TP (g/dl)	Alb (g/dl)	AAG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
SD rats	4.8±0.2	2.09±0.23	32.9±6.6	7.8±1.8	15.1±1.4
Analbuminemic rats	4.6±0.1	0.16±0.05[a]	21.4±3.1[a]	11.6±1.8[a]	39.5±2.6[a]

TP, Total protein; Alb, albumin; AAG, α_1-acid glycoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein. [a]Value is significantly different from that for SD rats ($p<0.05$).

Chromatographic separation used a gradient elution mode of water containing 0.1% formic acid (mobile phase A) and of acetonitrile containing 0.1% formic acid (mobile phase B). Linear gradient elution was employed with the ratios of A:B as follows: 0 min, 95:5; 1 min 95:5; 4 min, 20:80; and 6 min, 20:80. Equilibration time was 3 min. The total flow rate was 0.2 ml/min. Ionization of sunitinib was performed in the positive electrospray ionization mode.

These assays were shown to be linear for the concentrations tested with a correlation coefficient of 0.998. The within- and between-day coefficients of variation for these assays were less than 14%. The detection limit of sunitinib was 0.5 ng/ml in plasma and 0.1 ng/ml in 4.5% HSA, 0.1% AAG and saline.

Concentration of AAG in rat plasma were determined by rat α-1-acid glycoprotein ELISA Test Kit (Life Diagnostics, West Chester, PA, USA), which is based on enzyme immunoassay.

Concentrations of albumin and total protein in plasma were determined with the bromocresol green method and the biuret protein assay, respectively. Concentrations of high-density lipoprotein (HDL) and low-density lipoprotein (LDL) in plasma were determined with commercial kits, Cholestest N HDL and Cholestest LDL, respectively (Sekisui Medical Co., Tokyo, Japan).

Pharmacokinetic data analysis. Plasma concentration–time data for sunitinib in each rat after a single intravenous administration were analyzed individually using a non-compartmental model. The area under the plasma concentration-time curve (AUC) and the area under the first-moment curve (AUMC) were calculated by the trapezoidal method until the last measurable concentration in plasma and were extrapolated to infinity. The systemic clearance (CL_{SYS}) was calculated as dose/AUC. The mean residence time (MRT) was calculated as MRT=AUMC/AUC. The V_{SS} was calculated as $V_{SS}=CL_{SYS} \times MRT$. The pharmacokinetic parameters (CL_{SYSU}, V_{SSU} and MRT_U) for unbound sunitinib were estimated in the same manner as that for total sunitinib concentration, where the unbound concentration was calculated using the total plasma concentrations and unbound fraction obtained from the protein-binding experiments.

Statistical analysis. Results are expressed as the mean±standard deviation (SD) for the indicated numbers of experiments. Statistical differences between SD and analbuminemic rats were assessed by the unpaired Student's *t*-test, and *p*-values of less than 0.05 were taken as statistical significant. Correlations between the bound and unbound concentrations were determined by least-squares linear regression analysis. Statistical analysis was performed with software package SPSS for Windows version 11.0J (SPSS Inc., Chicago, IL, USA).

Results

Biochemical data for SD and analbuminemic rats. Biochemical data for SD and analbuminemic rats are summarized in Table I. The concentrations of albumin and AAG in analbuminemic rats were significantly lower than in SD rats. LDL and HDL were significantly higher in analbuminemic rats than in SD rats, although there was no significant difference in the total protein concentrations between SD and analbuminemic rats.

Plasma concentration–time curves of sunitinib after a single intravenous injection in SD and analbuminemic rats. Mean semi-logrithmic plasma concentration–time curves for sunitinib in SD and analbuminemic rats after a single intravenous injection (3 mg/kg) are illustrated in Figure 1. Sunitinib was bi-phasically eliminated from plasma after injection. Plasma concentrations at the early distribution phase (α-phase) after intravenous injection in analbuminemic rats were significantly lower than those in SD rats. The corresponding pharmacokinetic parameters of sunitinib are summarized in Table II. The CL_{SYS} and V_{SS} for sunitinib were significantly greater in analbuminemic rats. However, no significant difference in the half-life was observed between SD and analbuminemic rats.

Protein binding behavior of sunitinib in SD and analbuminemic rats. The plasma protein-binding profiles of sunitinib in SD and analbuminemic rats are illustrated in Figure 2. As shown in Figure 2, the relationship between concentrations of bound and unbound of sunitinib in SD and analbuminemic rats was linear and the unbound fraction of sunitinib in analbuminemic rats (0.110±0.015) was significantly larger than that in SD rats (0.062±0.016).

The mean plasma concentrations of unbound sunitinib, which were calculated by multiplying the total plasma concentration for each rat by the unbound fraction, were plotted against time. The mean plasma concentration–time profiles for unbound sunitinib in SD and analbuminemic rats are illustrated in Figure 3. As shown in Figure 3, no

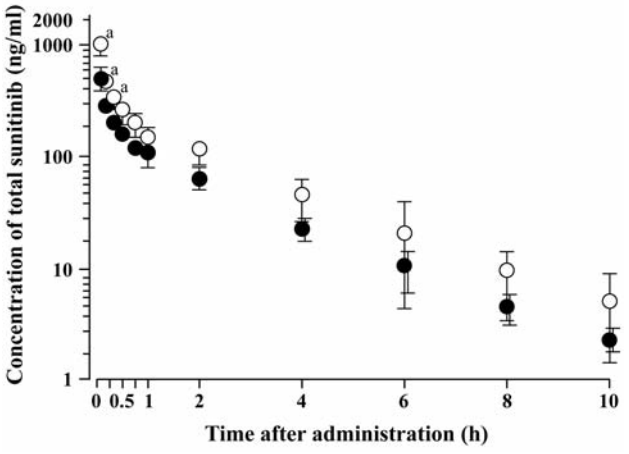

Figure 1. *Mean semi-logarithmic plasma concentration–time curves of sunitinib after a single intravenous administration (3 mg/kg) in Sprague-Dawley (SD) rats and analbuminemic rats. Each point represents the mean±SD (n=4-5). Open and closed circles represent SD and analbuminemic rats, respectively. [a]Significant difference between SD and analbuminemic rats at the early distribution phase (p<0.05). When the standard deviation is small, it is included in the symbol.*

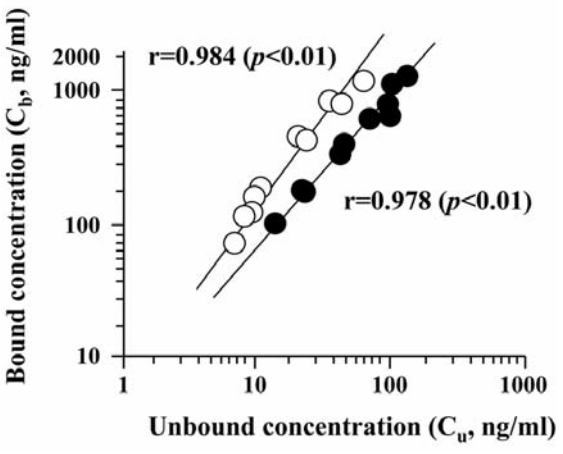

Figure 2. *Protein-binding profiles of sunitinib in fresh plasma obtained from Sprague-Dawley (SD) rats and analbuminemic rats. Each point represents data for sunitinib-spiked plasma samples at designated concentrations. Open and closed circles represent SD and analbuminemic rats, respectively. No concentration dependency in the protein-binding behavior of sunitinib was observed in either type of rat.*

Table II. *Pharmacokinetic parameters of total sunitinib after a single intravenous administration in Sprague-Dawley (SD) rats and analbuminemic rats.*

Animal	CL_{SYS} (L/h/kg)	V_{SS} (L/kg)	$t_{1/2}$ (h)
SD rats	1.26±0.32	2.37±0.25	1.74±0.34
Analbuminemic rats	2.17±0.26[a]	3.94±0.70[a]	1.62±0.08

Each value represents the mean±SD (n=4-5). CL_{SYS}, systemic clearance; V_{SS}, volume of distribution; $t_{1/2}$, elimination half-life. [a]Significantly different from SD rats (p<0.01).

Table III. *Pharmacokinetic parameters of unbound sunitinib after a single intravenous administration in Sprague-Dawley (SD) rats and analbuminemic rats.*

Animal	CL_{SYSU} (L/h/kg)	V_{SSU} (L/kg)	$t_{1/2U}$ (h)
SD rats	20.5±5.3	38.4±4.3	1.74±0.34
Analbuminemic rats	19.7±2.4	35.7±6.3	1.62±0.08

Each value represents the mean±SD (n=4-5). CL_{SYSU}, systemic clearance for unbound drug; V_{SSU}, volume of distribution for unbound drug; $t_{1/2U}$, elimination half-life for unbound drug. No significant differences in all pharmacokinetic parameters of sunitinib were observed between SD and analbuminemic rats.

significant differences in the concentration–time profiles for unbound sunitinib were observed between SD and analbuminemic rats. As shown in Table III, there were also no significant differences in the corresponding pharmacokinetic parameters of unbound sunitinib between SD and analbuminemic rats.

Protein-binding behavior of sunitinib to HSA and AAG. The protein-binding profiles for sunitinib in 4.5% HSA and 0.1% AAG are illustrated in Figure 4. As shown in Figure 4, significant linear relationships were observed between concentrations of bound and unbound sunitinib to HSA and AAG. Sunitinib also exhibited concentration-independent protein-binding profiles for both HSA and AAG. The binding potency of sunitinib to HSA and AAG was 65.3±4.6% and 33.7±6.3%, respectively, indicating that albumin exhibits higher affinity for sunitinib than does AAG.

Discussion

Sunitinib, which is widely used for the treatment of renal cell carcinoma and gastrointestinal stromal tumor, commonly causes various side-effects such as fatigue, nausea, diarrhea, anorexia, hypertension, and hand-foot syndrome. It is also known that a high plasma concentration of sunitinib uncommonly leads to severe damage to the liver. Therefore,

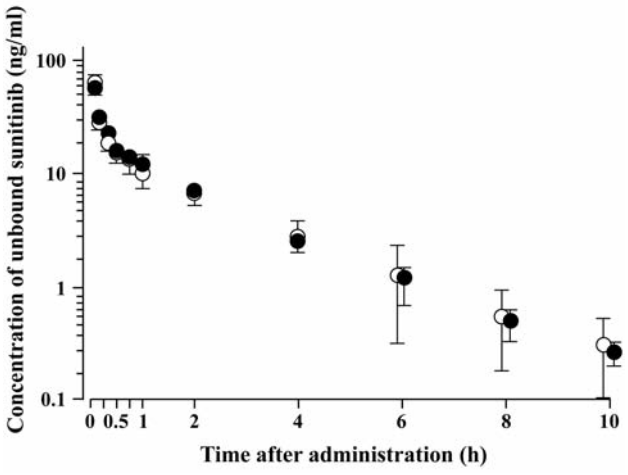

Figure 3. *Mean semi-logarithmic plasma concentration–time curves of unbound sunitinib after a single intravenous administration (3 mg/kg) in SD and analbuminemic rats. Each point represents the mean±SD (n=4-5). Open and closed circles represent SD and analbuminemic rats, respectively. No significant differences in plasma concentration data for unbound sunitinib at any sampling points were observed between SD and analbuminemic rats. When the standard deviation is small, it is included in the symbol.*

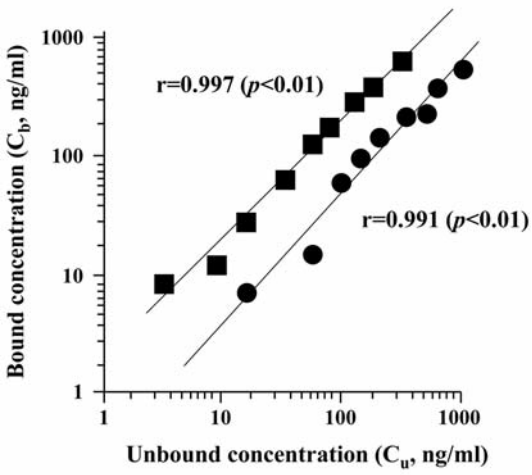

Figure 4. *Protein-binding profiles of sunitinib in 4.5% human serum albumin (HSA) and 0.1% α_1-acid glycoprotein (AAG). Each point represents data for sunitinib-spiked samples at designated concentrations. Closed squares and circles represent 4.5% HSA and 0.1% AAG, respectively. No concentration dependency in the protein-binding behavior of sunitinib in 4.5% HSA and 0.1% AAG was observed.*

the dosage adjustment of sunitinib, concomitant drugs, blood pressure and liver function should be routinely monitored because the incidence of such side-effects may be related to the pharmacokinetic behaviors of sunitinib (21). It is generally accepted that protein-binding plays a key role in determining the pharmacokinetics (distribution, metabolism and elimination) and the potency of the pharmacological effects of drugs. In the present study, we examined the contribution of plasma proteins, albumin and AAG, to the pharmacokinetics of sunitinib with high protein-binding potency in SD and analbuminemic rats.

Firstly, we investigated the comparative pharmacokinetics of sunitinib between SD and analbuminemic rats after a single intravenous injection. Regarding the pharmacokinetic parameters of sunitinib in SD rats in this study, the obtained CL_{SYS} (1.3 l/h/kg) and half-life (1.7 h) were slightly lower than those reported by Haznedar and colleagues (1.8 l/h/kg and 2.1 h, respectively) (22). However, the V_{SS} of sunitinib was 2.4 l/kg, which was approximately one-half of that reported by Haznedar and colleagues (5.5 l/kg). This discrepancy in the V_{SS} may be explained by differences in number of blood sampling points in the distribution phase (2, 15, 30 and 60 min after injection in their study) and the methods of intravenous injection (1-min infusion in their study) between ours and their study. In the present study, significant differences in the pharmacokinetic parameters of sunitinib, CL_{SYS} and V_{SS}, were observed between SD and analbuminemic rats. Namely, the two pharmacokinetic parameters CL_{SYS} and V_{SS} for sunitinib were significantly increased in analbuminemic rats compared with SD rats. A significant increase in the V_{SS} for total sunitinib observed in analbuminemic rats can be explained by the knowledge that the apparent volume of distribution generally increases as the concentration of the unbound drug in plasma increases.

It is reported elsewhere that the expression of hepatic cytochrome *P450 3a2* (Cyp3a2) (which corresponds to CYP3A4 in humans), which is closely related to the metabolism of sunitinib (3, 21, 23, 24), in analbuminemic rats is unchanged compared to SD rats (14). We also reported previously that there is no significant difference in the expression of hepatic P-glycoprotein (Abcb1), which is an efflux transporter affected by sunitinib (25, 26), between SD and analbuminemic rats (17). It is unlike that the Cyp3a2-mediated metabolism and P-glycoprotein-mediated excretion of sunitinib are altered in analbuminemic rats. These findings suggest that the increase in the CL_{SYS} of sunitinib is mainly due to the increase in V_{SS} because no significant difference in its half-life was observed between SD and analbuminemic rats. Consequently, the differences in the pharmacokinetics of sunitinib between SD and analbuminemic rats may be explained by differences in the protein-binding behaviors.

Plasma protein-binding is known to be a limiting factor in drug disposition, since only the unbound drug is capable of diffusing across biological membranes to be distributed into target organ tissues, as well as being subject to hepatic metabolism and renal excretion. It is reported that sunitinib

binds strongly to human and rat plasma proteins (approximately 95% and 99%, respectively) (21). Therefore, the protein-binding potency of sunitinib in analbuminemic rats is surmised to be lower than that of SD rats.

Secondly, we examined *in vitro* the protein-binding of sunitinib to plasma proteins in SD and analbuminemic rats using an equilibrium dialysis method because a preliminary *in vitro* protein-binding experiment using ultrafiltration method found considerable nonspecific binding of sunitinib to the filtration device. Expectedly, the protein-binding experiments showed that the extent of the protein-binding of sunitinib in analbuminemic rats was significantly less (89.0±1.5%) than that in SD rats (93.9±1.6%). The protein-binding profiles of sunitinib exhibited concentration-independency in the concentration range studied as seen in Figure 2, which was supported of Haznedar and colleagues (22). However, the protein-binding potency of sunitinib observed in SD rats in our study was lower than their reported value (22). Since it is not noted in the literature whether any of the equilibrium dialysis methods, ultrafiltration method or other methods were used for their protein-binding experiments, the discrepancy in the binding potency between our study and theirs may be explained by differences in the methodology of protein-binding experiments.

When the plasma concentrations of unbound sunitinib, as calculated by multiplying the total plasma concentration in each SD and analbuminemic rat by the unbound fraction (0.062 and 0.110, respectively), were plotted against time, no significant differences in the plasma concentration–time profiles and pharmacokinetic parameters of unbound sunitinib were observed between SD and analbuminemic rats. These results suggest that the increase in the V_{SS} of sunitinib observed in analbuminemic rats can be explained by the lower protein-binding potency in analbuminemic rats compared to SD rats.

In the present study, the concentration of AAG in analbuminemic rats was found to be significantly lower than that in SD rats, although the concentration of AAG in SD and analbuminemic rats was reported to be almost the same by Emori and colleagues (27). We have already reported that plasma concentration of total protein in SD and analbuminemic rats is almost the same, but those of total cholesterol, LDL and HDL in analbuminemic rats are significantly higher than that in SD rats (17).

When protein-binding of albumin and AAG to sunitinib were measured, it was unexpectedly found that the binding potency of AAG was low (34%), suggesting that AAG has low affinity for this basic drug. On the other hand, binding of albumin was moderate (67%), suggesting that it exhibits moderate affinity for sunitinib. On the basis of these findings, we assume that the lower protein-binding potency observed in analbuminemic rats may have been caused mainly by the lower albumin concentration, although we cannot exclude the possible contribution of other plasma proteins, such as lipoproteins and globulins.

In conclusion, the present study using SD and analbuminemic rats clearly demonstrated that the pharmacokinetics of sunitinib is altered by changes in concentrations of plasma proteins, including albumin and AAG. We can conclude that changes in the concentration of albumin play a more important role in the pharmacokinetics of sunitinib rather than changes in AAG concentration. Although it is known that there is high inter-patient and intra-patient variability of AAG concentration in patients with cancer (28), it is considered that there is no need to monitor AAG concentration in those patients with cancer who are scheduled to receive sunitinib therapy.

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