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Monitoring Serum Levels of Sorafenib and Its *N*-Oxide Is Essential for Long-Term Sorafenib Treatment of Patients with Hepatocellular Carcinoma

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Sorafenib, an oral multi-kinase inhibitor, is the final therapy prior to palliative care for advanced hepatocellular carcinoma (HCC). However, due to its adverse effects, 20% of patients must discontinue sorafenib within 1 month after first administration. To identify ways to predict the adverse effects and administer the drug for longer periods, we explored the relationship between the duration of sorafenib treatment and the pharmacokinetics of sorafenib and its major metabolite, sorafenib *N*-oxide. Twenty-five subjects enrolled in the study were divided into two groups: patients with dosage reduced or withdrawn due to adverse effects (n = 8), and patients with dosage maintained for 1 month after initial administration (n = 17). We evaluated early sorafenib accumulation as the area under the curve of sorafenib and sorafenib *N*-oxide concentrations during days 1-7 ($AUC_{sorafenib}$ and $AUC_{N\text{-oxide}}$, respectively). Inter-group comparison revealed that $AUC_{N\text{-oxide}}$ and AUC ratio ($AUC_{N\text{-oxide}}$ /$AUC_{sorafenib}$) were significantly higher in the dosage reduction/withdrawal group (P = 0.031 and P = 0.0022, respectively). Receiver operating characteristic analysis indicated that $AUC_{N\text{-oxide}}$ and AUC ratio were reliable predictors of adverse effects. When patients were classified by cut-off points ($AUC_{N\text{-oxide}}$: 2.0 μg·day/mL, AUC ratio: 0.13), progression-free survival was significantly longer in patients with $AUC_{N\text{-oxide}} \leq 2.0$ μg·day/mL (P = 0.0048, log-rank test). In conclusion, we recommend to simultaneously monitor serum levels of sorafenib and its *N*-oxide during the early stage after the first administration, which enables us to provide safe and long-term therapy for each HCC patient with sorafenib.

Keywords: drug-induced toxicity; hepatocellular carcinoma; sorafenib; sorafenib *N*-oxide; therapeutic drug monitoring
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Introduction

Sorafenib is the world's first oral molecularly targeted drug approved for treatment of patients with advanced or metastatic renal-cell carcinoma (Wilhelm et al. 2006). The drug was approved in the U.S.A. in December 2005. In Japan, sorafenib was approved in January 2008 for the same indications, and subsequently approved in May 2009 for treatment of patients with hepatocellular carcinoma (HCC) judged to be unresectable or refractory to transcatheter arterial chemoembolization (TACE) and radiofrequency ablation (RFA). In June 2014, the drug was approved for the treatment of patients with locally advanced or metastatic differentiated thyroid cancer (http://www.info.pmda.go.jp/go/interview/1/630004_4291017F1025_1_1F).

Because sorafenib is the final therapy prior to palliative care for HCC patients, it is important that administration be continued as long as possible. However, according to the second interim report of Nexbar® Special Drug Use Surveillance for HCC (Bayer Yakuhin, Osaka, Japan),

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adverse effects force 20% of patients to discontinue sorafenib within 1 month after the first administration. Diarrhea, rash, fatigue, hand-foot skin reactions, and hypertension are the most common adverse events associated with sorafenib. Serious adverse effects such as liver failure, hepatic encephalopathy, and pneumonitis also arise in some cases (http://www.nexavar.jp/unmember/pdf/hcc201205.pdf).

Sorafenib is absorbed from the gastrointestinal tract and reaches the liver via the portal vein. Therefore, its bioavailability is influenced by the activity of the excretory transporters ABCB1 and ABCG2 in the small-intestinal mucosa (Hu et al. 2009; Gnoth et al. 2010). In addition, sorafenib is primarily metabolized by cytochrome P450 3A4 (CYP3A4) in the small-intestinal mucosa or the liver, and it is also subjected to glucuronidation mediated by uridine diphosphate glucuronosyl transferase (UGT) 1A9 (Lathia et al. 2006; Peer et al. 2012; Filppula et al. 2014).

Sorafenib and its metabolites are predominantly passed in the feces, whereas a portion of the glucuronide is broken down to sorafenib by β-glucuronidase expressed by bacterial flora in the intestine. Subsequently, sorafenib undergoes enterohepatic circulation and reaches steady state within 7-10 days after administration is initiated (http://www.info.pmda.go.jp/go/interview/1/630004_4291017F1025_1_1F; van Erp et al. 2009). According to a safety information letter from the Ministry of Health, Labour and Welfare of Japan (November 18, 2009), signs of serious adverse effects are observed in patients' laboratory results within 1 week after the first administration (http://www.mhlw.go.jp/file/06-Seisakujouhou-11120000-Iyakushokuhinkyoku/0000076332.pdf). Together, these observations suggest that knowledge of the pharmacokinetics of sorafenib might be useful in avoiding adverse effects during the early stages of administration of this drug.

Recently, Blanchet et al. (2009) reported that the plasma concentration of sorafenib in patients with adverse effects (grade > 3) is 1.5-fold higher than in patients with no adverse effects. Boudou-Rouquette et al. (2012) also reported that the AUC of sorafenib is associated with the highest risk of developing any type of grade ≥ 3 toxicity.

Therefore, we started therapeutic drug monitoring of the trough levels of sorafenib and its major metabolite, sorafenib *N*-oxide, during hospital stays and in the outpatient clinic. In this study, we investigated the association between adverse effects and the accumulation of sorafenib and its *N*-oxide, with the goal of providing safe and long-term sorafenib therapy for patients with HCC.

Human Subjects and Methods

Human subjects

This research was conducted as an observational study. The institutional committee of the Graduate School of Medicine at Tohoku University approved the use of human subjects in this study. Twenty-five HCC patients treated with sorafenib at Tohoku University Hospital were enrolled. Patients provided written informed consent according to the protocol adopted by the institutional review board of the Graduate School of Medicine at Tohoku University in February 2011. Characteristics of these patients undergoing sorafenib treatment are provided in Table 1. The first dose of sorafenib was determined by the attending doctor based on the hepatic functional reserve of each patient. When the frequency of administration changed to once per day, sorafenib was taken after breakfast.

Dosage-reduction or withdrawal criteria for sorafenib administration

Sorafenib-related adverse effects were graded according to the National Cancer Institute Common Terminology Criteria for adverse events, version 4.0. The reference ranges of neutrophil counts, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in Tohoku University Hospital were 1.64-5.95 × 10^3 cells/μL, 8-38 IU/L, 4-43 IU/L, and 115-330 IU/L, respectively. The attending doctor made decisions about dosage reduction or withdrawal of sorafenib treatment according to the criteria described in the package insert.

Blood sampling

Peripheral blood samples (2 ml each) were collected in non-coated blood collection tubes. For hospitalized patients, samples were taken at trough in the morning (AM 6:00) every other day during the hospital stay. For outpatients, samples were taken every 2-4 weeks during hospital visits (AM 7:30-8:30), along with routine biochemical blood tests. After blood collection, while waiting to see the doctor, outpatients ate breakfast, followed by administration of sorafenib. After centrifugation of the blood collection tubes, serum samples were obtained and analyzed following the protocols described below. Residual serum was stored at −80°C.

Preparation of analytical samples

First, 200 μL of IS (internal standard) solution (5 μg/mL) was added to 200 μL of serum in a glass tube. After mixing for 10 s, the tube was centrifuged at 1,580 *g* for 10 min. An aliquot (300 μL) of the supernatant was transferred into a plastic tube (1.5 mL) containing 75 μL of deionized purified water. Next, 250 μL of the resulting solution was applied to a Bond Elut C18 column (100 mg/1 mL, Agilent Technologies, Inc., Santa Clara, CA, USA), which was washed with 600 μL of 40% acetonitrile. The analytes were eluted with 1,000 μL of acetonitrile, and the eluate was evaporated to dryness under a nitrogen gas stream. The residue was reconstituted in 100 μL of 20 mM ammonium acetate buffer (pH 4.0)/acetonitrile (30:70, v/v). An aliquot (5 μL) was analyzed by HPLC. Details were described in a previous report (Shimada et al. 2014).

Determination of sorafenib and its major metabolite, sorafenib N-oxide

Sorafenib and sorafenib *N*-oxide were determined simultaneously using a liquid chromatography system (Agilent 1100) consisting of a pump with degas option, autosampler, and UV detector (Agilent Technologies, Inc.). Chromatographic separation was achieved on an Inertsil ODS-3 column (2.1 mm i.d. × 150 mm, 5 μm, GL Science, Inc., Tokyo, Japan) associated with a guard column packed with the same material. The composition of the mobile phase was 20 mM ammonium acetate buffer (pH 4.0)/acetonitrile (30:70, v/v). The flow rate was 200 μL/min throughout the 15-min run. The eluent was monitored at a wavelength of 265 nm. The intra-assay and inter-assay coefficients of variation and accuracy bias were less than 15% (Shimada et al. 2014).

Evaluation of cumulative sorafenib and sorafenib N-oxide

The cumulative area under the concentration-time curve (AUC) between day 1 and day 7 was calculated according to the trapezoidal rule using the Excel 2010 software (Microsoft, Redmond, WA, USA). AUC of sorafenib and sorafenib N-oxide during days 1-7 were described as $AUC_{sorafenib}$ and $AUC_{N\text{-oxide}}$, respectively. $AUC_{N\text{-oxide}}/AUC_{sorafenib}$ was described as the AUC ratio.

Performance evaluation

Receiver Operating Characteristic (ROC) curves were constructed using the $AUC_{N\text{-oxide}}$ or AUC ratio, as well as adverse effects resulting in dosage reduction or withdrawal, from 25 patients with HCC (Zweig and Campbell 1993; Muller et al. 2007; Harmon et al. 2011). The area under the ROC curves (AUC_{ROC}) was calculated, and the statistical significance was tested using nonparametric assumptions. ROC curves can reveal the efficacy of a test by providing information about both sensitivity and specificity at different cut-off points. Sensitivity and specificity measure the ability of a test to distinguish true and false positives within a dataset. Kaplan-Meier analysis was performed to compare the progression-free survival period (PFSP) between patients with $AUC_{N\text{-oxide}} \leq 2.0$ μg·day/mL and those with $AUC_{N\text{-oxide}} > 2.0$ μg·day/mL. Cox proportional hazards models were used to examine the effects of $AUC_{N\text{-oxide}} > 2.0$ μg·day/mL and AUC ratio > 0.13 on the duration of treatment with sorafenib. The results are reported as risk ratio with 95% CI.

Statistical analysis

Comparisons of means of the two groups (dosage reduced or withdrawn vs. dosage maintained) were performed using the independent samples Mann-Whitney U test. *P* values less than 0.05 were considered to represent statistically significant differences. All statistical analysis and figure preparation was performed using the Excel 2010 software. Kaplan-Meier and Cox proportional hazards model analyses were performed using JMP Pro (ver.11.0; SAS Institute, Cary, NC, USA).

Results

Association between serum sorafenib concentration and adverse effects

Hand-foot skin reaction (grade 2) and intense lassitude were observed in an HCC patient treated with the recommended daily dose of sorafenib (400 mg b.i.d.) at an outpatient clinic 56 days after the first administration. The attending doctor elected to give a smaller dose (400 mg once daily after breakfast). After 2 weeks (70 days after initiation of administration), the severity of the skin reaction was reduced. In this patient, the serum concentration of sorafenib was 2.6 μg/mL at discharge (day 9), 6.1 μg/mL on day 56, and 4.1 μg/mL on day 70 (Fig. 1). These results suggest that serum sorafenib concentration is associated with the drug's adverse effects.

Patient status before and after taking sorafenib

Sera from 25 patients taking sorafenib were obtained immediately before the first administration (1 day), and then blood samples were collected at least three times within the next 7 days, during hospitalization. Characteristics of these patients are shown in Table 1. For patients who experienced a dosage reduction or withdrawal within 1 month of initiating sorafenib treatment, the details reasons are provided in Table 2. The adverse reactions were classified as follows: skin disorder (No. 2, hand-foot skin reaction [grade 2]; No. 5, facial and cervical erythema [grade 1]; No. 6, erythema multiform drug eruption [grade 3]; No. 11, hand-foot skin reaction [grade 2], acneiform drug eruption, and suspicion of maculopapular drug eruption [grade 3]; No. 21, hand-foot skin reaction [grade 2] accompanied by cellulitis; No. 24, hand-foot skin reaction [grade 2]); neu-

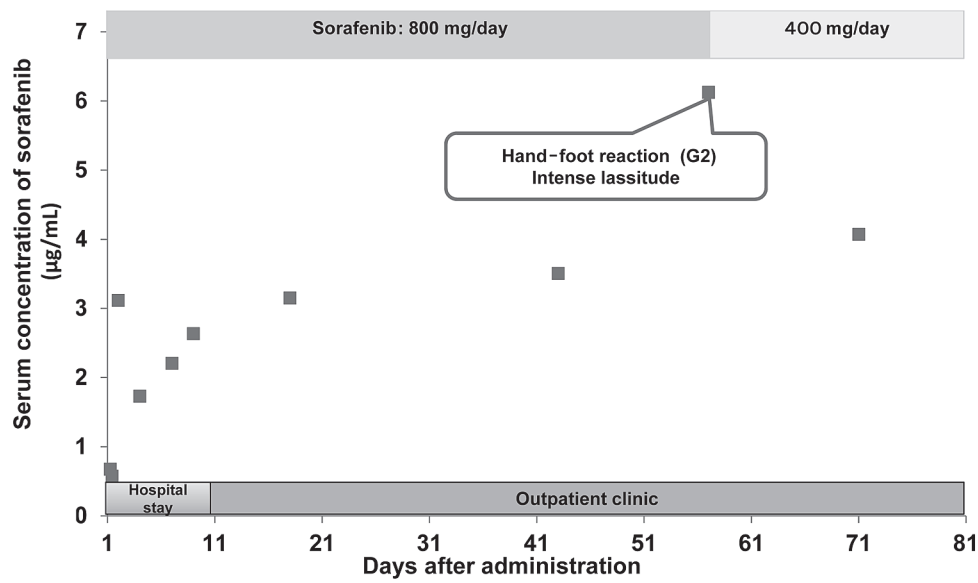

Fig. 1. Monitoring of serum trough concentration of sorafenib in a patient after initiation of treatment. Closed squares (■) show serum concentrations of sorafenib at trough in the morning. Hand-foot reaction (G2) and intense lassitude were observed on day 56 after initial administration of sorafenib.

Table 1. Characteristics of patients undergoing sorafenib treatment.

Characteristic	n = 25
Age (years)	
Median	65
Range	56-85
Sex	
Male	20 (80%)
Female	5 (20%)
Child-Pugh	
A	23 (92%)
B	2 (8%)
Hepatitis virus	
None	8 (32%)
B	7 (28%)
C	10 (40%)
Dosage	
Dosage maintenance	17 (68%)
Dosage reduction or withdrawn (≤ 1 month)	8† (32%)

Except for Age, data are shown as n (%).
†Major reasons for dosage reduction or withdrawal included skin disorder, hepatic dysfunction, and neutropenia.

tropenia (neutrophil count: No. 5, 1.01 (day 1) → 0.78 (day 17); No. 24, 5.65 (day 1) → 0.83 (day 13); all units are $\times 10^3$ cells/μL); deterioration in liver function (No. 9, AST: 147→605, ALT: 205→304, ALP: 873→802; No. 14, AST: 25→112, ALT: 12→104, ALP: 433→948; all units are IU/L); and gastrointestinal disorder (No. 5, dysphagia with pain during swallowing [grade 2]).

$AUC_{sorafenib}$, $AUC_{N\text{-}oxide}$, and AUC ratio in patients taking sorafenib

We calculated $AUC_{sorafenib}$ and $AUC_{N\text{-}oxide}$ from day 1 to day 7. The AUC ratio ($AUC_{N\text{-}oxide}/AUC_{sorafenib}$) was also calculated during the same interval. Individual patients' AUC data are also shown in Table 2. We observed a greater than 10-fold inter-individual difference in the values of $AUC_{sorafenib}$, $AUC_{N\text{-}oxide}$, and AUC ratio among 22 patients, despite the fact that the same dosages (800 mg/day) were administered.

When we examined the reciprocal relationship among $AUC_{sorafenib}$, $AUC_{N\text{-}oxide}$, and AUC ratio, we found that the decision coefficients (r^2) of $AUC_{N\text{-}oxide}$ vs. $AUC_{sorafenib}$, $AUC_{N\text{-}oxide}$ vs. AUC ratio, and $AUC_{sorafenib}$ vs. AUC ratio were 0.82 ($P < 0.0001$), 0.42 ($P < 0.0004$), and 0.095 ($P = 0.1347$), respectively (Fig. 2).

Correlation between adverse effects and $AUC_{sorafenib}$, $AUC_{N\text{-}oxide}$, and AUC ratio

We compared $AUC_{sorafenib}$, $AUC_{N\text{-}oxide}$, and AUC ratio between a group that underwent dosage reduction or withdrawal (8 patients) and a group in which dosage was maintained for 1 month (17 patients). Statistical comparisons were performed using the Mann-Whitney U test. As shown in Fig. 3, $AUC_{sorafenib}$ was higher in the dosage reduction/withdrawal group than in the dosage maintenance group, although statistical comparison revealed no significant differences ($P = 0.19$). $AUC_{N\text{-}oxide}$ and AUC ratio were significantly higher in the dosage reduction/withdrawal group ($AUC_{N\text{-}oxide}$: $P = 0.031$, AUC ratio: $P = 0.0022$).

Accuracy of $AUC_{N\text{-}oxide}$ and AUC ratio as predictive factors

In light of the association between $AUC_{N\text{-}oxide}$ or AUC ratio and dose reduction or withdrawal within 1 month, we performed ROC curve analysis. The $AUC_{N\text{-}oxide}$ cut-off for response discrimination was determined from the point on the ROC curve with the minimum distance from the point corresponding to sensitivity and specificity values of 1.0 (Schisterman et al. 2005) (Fig. 4). The calculated cutoff values for $AUC_{N\text{-}oxide}$ and AUC ratio were 2.0 μg·day/mL and 0.13, respectively. In general, prediction accuracy was evaluated using the AUC_{ROC}. AUC_{ROC} of $AUC_{N\text{-}oxide}$ and AUC ratio were 0.76 and 0.86, respectively. These results suggest that, compared to $AUC_{N\text{-}oxide}$, AUC ratio is a superior predictor of adverse effects within 1 month after initial administration of sorafenib. Using these cut-off values, sensitivity and specificity of $AUC_{N\text{-}oxide}$ were 0.88 and 0.76, respectively, whereas sensitivity and specificity of AUC ratio were 1.0 and 0.59, respectively.

Elimination half-life ($t_{1/2}$) values of sorafenib and sorafenib N-oxide during the washout period in the patients taking sorafenib

To determine the relationship between adverse effects and accumulation of sorafenib, we calculated the $t_{1/2}$ values during the washout period after treatment was abandoned due to adverse effects. Two patients presented with both neutropenia and skin disorder (No. 5 and No. 24). The $t_{1/2}$ values of sorafenib and sorafenib *N*-oxide during the washout period were 15 h and 20 h, respectively, in patient No. 5, and 16 h and 22 h, respectively, in patient No. 24. On the other hand, the $t_{1/2}$ values of sorafenib and sorafenib *N*-oxide in two patients with skin disorders alone were 10 h and 8.6 h, respectively, in patient No. 6, and 11 h and 12 h, respectively, in patient No. 11. Based on these calculations, the $t_{1/2}$ values of sorafenib and sorafenib *N*-oxide were 1.5- and 2-hold higher, respectively, in patients with both neutropenia and skin disorder than in patients with skin disorders alone.

Associations of $AUC_{N\text{-}oxide}$ and AUC ratio with progression-free survival period of sorafenib treatment

Of the 25 patients enrolled in this study, eight who underwent dosage reduction or withdrawal for l month due to adverse effects, six (Nos. 1, 4, 8, 12, 13, 22) who transferred to their nearest hospital, and one (No. 20) who withdrew were counted as "censored". In other patients, we defined the date when their condition became exacerbated

Table 2. Initial dose, pharmacokinetic parameters, day of cessation of treatment, and adverse effects causing dose reduction or withdrawal in HCC patients taking sorafenib.

Patient number	Initial dose (mg/day)	$AUC_{sorafenib}$ (μg • day/mL)	$AUC_{N\text{-oxide}}$ (μg • day/mL)	AUC ratio	Dose reduction or withdrawal within 1 month	
					Date	Adverse effect
1	800	18	1.4	0.078		
2	400	33	8.0	0.24	8	Skin disorder: HFSR (G2)
3	800	50	9.9	0.20		
4	800	12	1.5	0.13		
5	800	19	4.6	0.24	21	Skin disorder: facial and cervical erythema (G1); Dysphagia with pain during swallowing (G2); Neutropenia [neutrophil count: 1.01 (day 1) → 0.78 (day 17)]
6	800	31	6.0	0.19	13	Skin disorder: erythema multiforme drug eruption (G3)
7	800	29	6.7	0.23		
8	800	10	1.8	0.18		
9	800	23	3.8	0.17	5	Deterioration in liver function (AST: 147→605, ALT: 205→304, ALP: 873→802)
10	800	11	0.78	0.071		
11	800	22	6.0	0.27	11	Skin disorder: HFSR (G2), acneiform drug eruption, suspicion of maculopapular drug eruption (G3)
12	200	5.3	0.24	0.045		
13	800	23	3.8	0.17		
14	800	4.6	0.64	0.14	23	Deterioration in liver function (AST: 25→112, ALT: 12→104, ALP: 433→948)
15	400	9.5	0.85	0.090		
16	800	9.3	1.4	0.15		
17	800	5.4	0.65	0.12		
18	800	5.9	0.76	0.13		
19	800	7.3	0.63	0.086		
20	800	9.3	0.86	0.092		
21	800	7.6	2.1	0.28	9	Skin disorder: HFSR (G2) accompanied by cellulitis
22	800	8.1	1.0	0.12		
23	800	15	3.8	0.25		
24	800	13	6.1	0.47	13	Skin disorder: HFSR (G2) Neutropenia [neutrophil count: 5.65 (day 1) → 0.83 (day 13)]
25	800	8.6	2.0	0.23		

Shading indicates appearance of adverse effects leading to dosage reduction or withdrawal within 1 month after administration. Underlined values are greater than the cutoff point. Adverse effects observed on the day of cessation of sorafenib treatment are shown. Changes in the value of AST (IU/L), ALT (IU/L), and ALP (IU/L) between day 1 and the termination of administration in patients with deterioration in liver function leading to withdrawal are shown. Neutrophil counts are shown as $\times 10^3$ cells/μL.

as the day when they were immediately hospitalized or died due to aggravation of primary disease or overall status (Nos. 3, 10, 15, 17, 18, 19, 23, 25), and complained of intense lassitude (Nos. 7, 16).

Therefore, we divided 25 patients into two groups according to the cutoff points of $AUC_{N\text{-oxide}}$ (2.0 μg·day/mL) or AUC ratio (0.13). The median PFSP in the patients with $AUC_{N\text{-oxide}} \leq 2.0$ μg·day/mL (n = 14, 7 patients censored) and that with $AUC_{N\text{-oxide}} > 2.0$ μg·day/mL (n = 11, 8 patients censored) were 380 (95% CI, 207-610) and 150 (95% CI, 142-152) days, respectively (Table 3); the median PFSP in patients with AUC ratio ≤ 0.13 (n = 10, 5 patients censored) and that with AUC ratio > 0.13 (n = 15, 10 patients censored) were 380 (95% CI, 184-839) and 150 (95% CI, 144-276) days, respectively. When patients were grouped by their $AUC_{N\text{-oxide}}$ and AUC ratio values, Kaplan-Meier analysis revealed a statistically significant difference in PSFP between patients with $AUC_{N\text{-oxide}} \leq 2.0$ μg·day/mL and those with $AUC_{N\text{-oxide}} > 2.0$ μg·day/mL (log-rank test; $P = 0.0048$) (Fig. 5A). PFSP did not differ significantly between patients with AUC ratio ≤ 0.13 and patients with AUC ratio > 0.13 (log-rank test; $P = 0.1248$) (Fig. 5B).

We next examined the effects of $AUC_{N\text{-oxide}} > 2.0$ μg·day/mL and AUC ratio > 0.13 on PFSP using the Cox

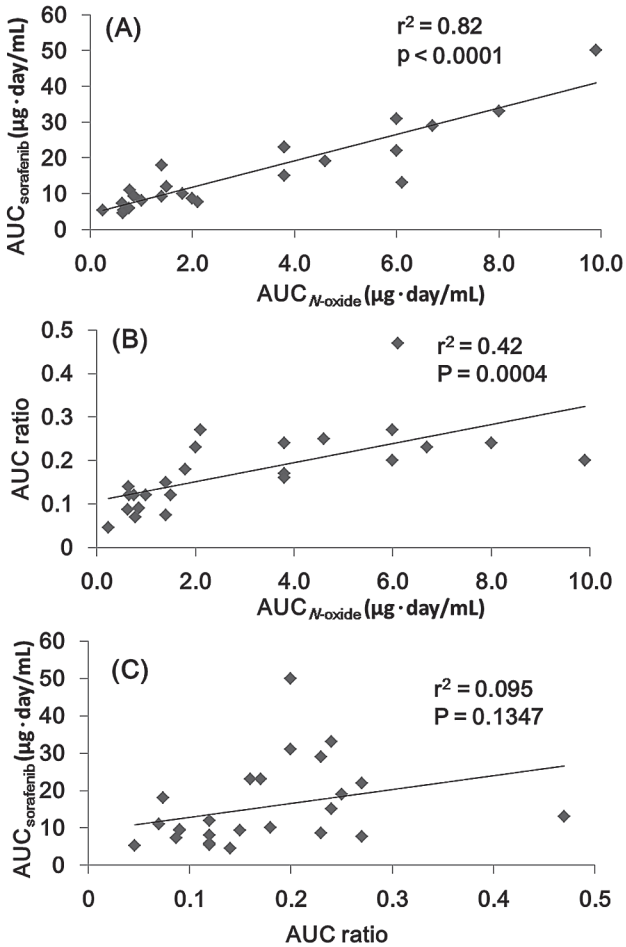

Fig. 2. Reciprocal relationships among $AUC_{sorafenib}$, $AUC_{N\text{-oxide}}$, and AUC ratio.
The following correlations are shown for 25 subjects: (A) $AUC_{N\text{-oxide}}$ (X-axis) vs. $AUC_{sorafenib}$ (Y-axis); (B) $AUC_{N\text{-oxide}}$ (X-axis) vs. AUC ratio (Y-axis); and (C) AUC ratio (X-axis) vs. $AUC_{sorafenib}$ (Y-axis).

proportional hazards model (Table 2). When patients were grouped according to their $AUC_{N\text{-oxide}}$, PFSP was significantly longer for patients with $AUC_{N\text{-oxide}} \leq 2.0$ μg·day/mL than for patients with $AUC_{N\text{-oxide}} > 2.0$ μg·day/mL (risk ratio, 0.081; 95% CI, 0.0040-0.64; *P* (Prob>Chisq) = 0.0173). When patients were grouped according to their AUC ratio, PFSP did not differ significantly between patients with AUC ratio ≤ 0.13 and patients with AUC ratio > 0.13 (risk ratio, 0.39; 95% CI, 0.11-1.4; *P* (Prob>Chisq) = 0.1534).

In patients with treatment durations greater than 6 months, the values of $AUC_{sorafenib}$, $AUC_{N\text{-oxide}}$, and AUC ratio were 7.7 ± 1.9 μg·day/mL, 1.13 ± 0.56 μg·day/mL, and 0.14 ± 0.054, respectively. From the corresponding AUC values, the trough levels of sorafenib and sorafenib *N*-oxide were back-calculated as approximately 2.0 and 0.28 μg/mL, respectively.

Discussion

To continue treatment of patients with molecularly targeted drugs for extended periods of time, it is preferred to identify the predictors for adverse effects of these drugs. To date, data regarding determinants of sorafenib-induced toxicity remain scarce. Studies to data have reported an association of age and therapy discontinuation in Japanese patients (Morimoto et al. 2011); associations of cumulative sorafenib dose, Eastern Cooperative Oncology Group (ECOG) PS, and female gender with hand-foot skin reaction (Azad et al. 2009; Dranitsaris et al. 2012); and an association of UGT1A9-2152 T allele with grade ≥ 2 diarrhea (Boudou-Rouquette et al. 2012). To determine the effect of drug accumulation on drug-induced toxicity in the context of clinical use of sorafenib, AUC analysis must be applied. While monitoring the serum level of sorafenib in one patient, we observed an adverse effect, hand-foot skin reaction (G2), accompanied by an increase in the trough

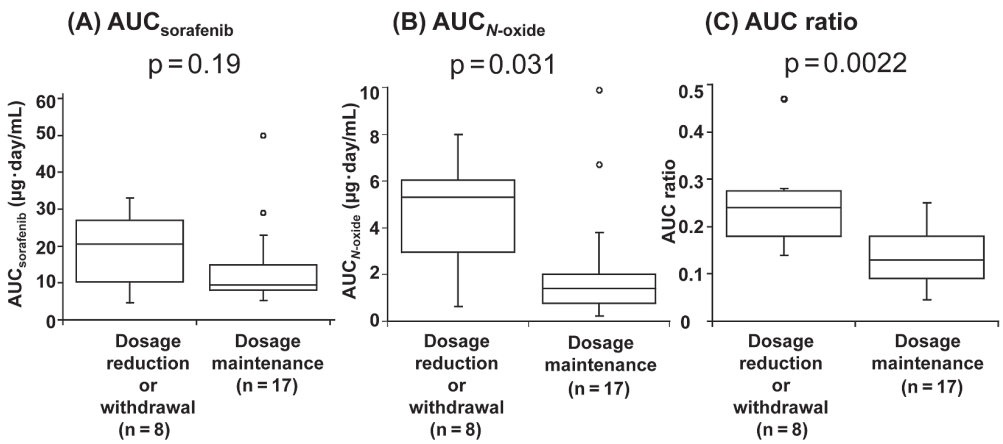

Fig. 3. Comparisons of $AUC_{sorafenib}$, $AUC_{N\text{-oxide}}$, and AUC ratio between dosage-reduced/withdrawn and dosage-maintained groups.
(A) $AUC_{sorafenib}$, (B) $AUC_{N\text{-oxide}}$, and (C) AUC ratio ($AUC_{N\text{-oxide}}/AUC_{sorafenib}$) in dosage-reduced/withdrawn (n = 8) and dosage-maintained (n = 17) groups. Boxes indicate median values, and the ends of the vertical lines show minimum and maximum values. The bottoms and tops of the boxes represent the first and third quartiles. Outliners are represented by ○. Comparisons of quartiles between the two groups were performed using the Mann-Whitney U test. *P* values less than 0.05 were considered to represent statistically significant differences.

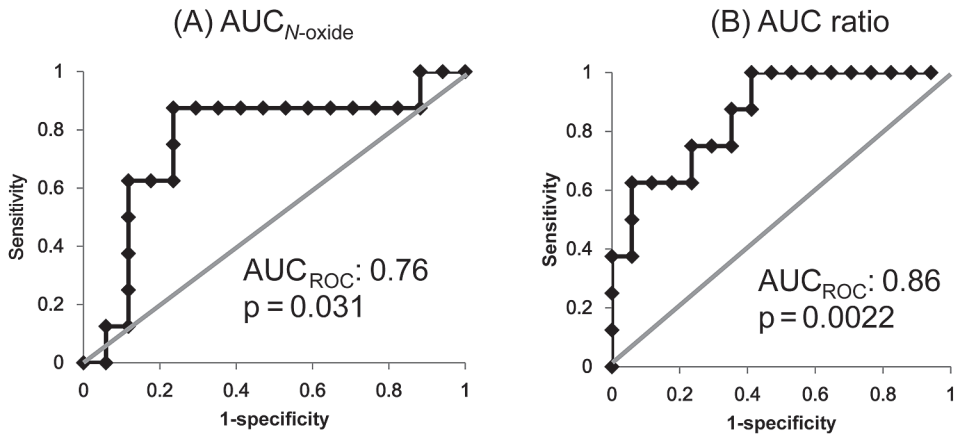

Fig. 4. Receiver operating characteristic (ROC) curve analysis to evaluate $AUC_{N\text{-oxide}}$, and AUC ratio as predictors of adverse effects in HCC patients taking sorafenib.
ROC curves of (A) $AUC_{N\text{-oxide}}$ and (B) AUC ratio are shown. AUC_{ROC} and two-tailed *P* values are provided in each case.

Table 3. Cox regression analysis of progression-free survival period in patients grouped according to $AUC_{N\text{-oxide}}$ andAUC ratio.

Patient group	n	censored	Median of PFSP (days)	Risk ratio	95% CI	*P* value
$AUC_{N\text{-oxide}}$ (μg • day/mL)						
G1: ≤ 2.0	14	7	380	–	207-610	} log-rank: 0.0048
G2: > 2.0	11	8	150	–	142-152	
G2/G1				12	1.6-250	0.0173
G1/G2				0.081	0.00040-0.64	0.0173
AUC ratio						
G1: ≤ 0.13	10	5	380	–	184-839	} log-rank: 0.1248
G2: > 0.13	15	10	150	–	144-276	
G2/G1				2.5	0.69-9.4	0.1534
G1/G2				0.39	0.11-1.4	0.1534

G, group; PFSP, progression-free survival period.

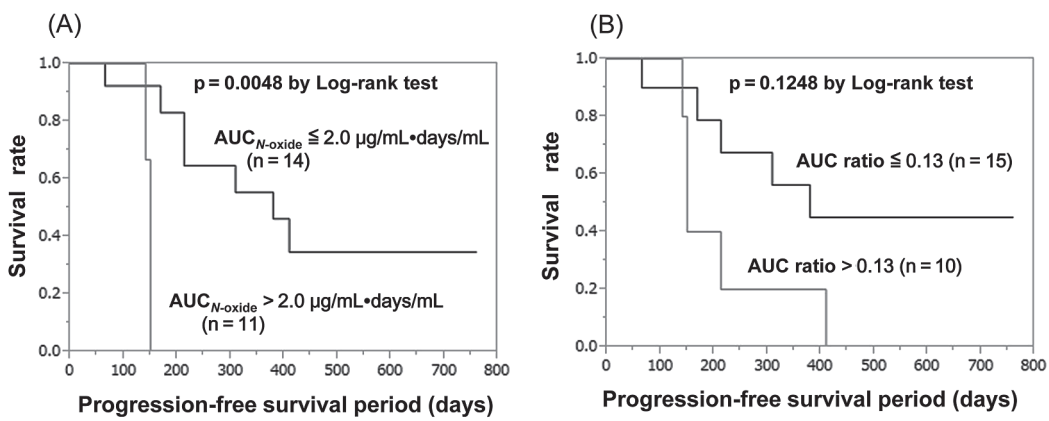

Fig. 5. Kaplan-Meier estimates of progression-free survival rates in patients with HCC taking sorafenib, grouped according to $AUC_{N\text{-oxide}}$ and AUC ratio.
Patients were grouped according to (A) $AUC_{N\text{-oxide}}$ or (B) AUC ratio. A log-rank test revealed statistically significant differences in survival rates between patients with $AUC_{N\text{-oxide}} > 2.0$ and those with $AUC_{N\text{-oxide}} \leq 2.0$ ($P = 0.0048$).

sorafenib level. This observation was similar to those in other reports (Blanchet et al. 2009; Boudou-Rouquette et al. 2012) and suggests that sorafenib-related adverse effects are correlated with serum concentration of the drug.

Therefore, we ascertained the association between dosage reduction/withdrawal of sorafenib and the AUC of sorafenib or sorafenib *N*-oxide during days 1-7 in 25 patients with HCC. We showed for the first time that the AUC of the sorafenib *N*-oxide concentration ($AUC_{N\text{-oxide}}$) during days 1-7 and the $AUC_{N\text{-oxide}}/AUC_{sorafenib}$ ratio during the same interval are determinants of the development of adverse effect leading to dosage reduction or withdrawal, and that $AUC_{N\text{-oxide}}$ and AUC ratio are predictive of adverse effects.

Boudou-Rouquette et al. (2012) found that AUC of sorafenib during days 1-30 was associated with the occurrence of any type of grade ≥ 3 toxicity, whereas we did not observe that the AUC of sorafenib during days 1-7 was significantly higher in the dosage reduction/withdrawal group than in the dosage maintenance group. We observed a good correlation between $AUC_{sorafenib}$ and $AUC_{N\text{-oxide}}$ ($r^2 = 0.82$, $P < 0.0001$, Fig. 2A), suggesting that the serum concentration of *N*-oxide increased along with the concentration of sorafenib within the observed concentration range. It is possible that a larger population analysis might reveal that $AUC_{sorafenib}$ is also a predictor of adverse effects.

Although only 25 patients enrolled in this study, we were able to use ROC analysis to detect an association between the pharmacokinetics of sorafenib and its *N*-oxide and adverse effects occurring within the first month of sorafenib therapy. The AUC_{ROC} of $AUC_{N\text{-oxide}}$ and AUC ratio were 0.76 and 0.86, respectively, indicating that these predictors were of moderate accuracy.

However, the detailed mechanism underlying the association between the AUC ratio or $AUC_{N\text{-oxide}}$ and the occurrence of adverse effects remains obscure. According to the Nexavar® interview form, the geometric average for AUC of [^{14}C]sorafenib in male rat skin is 86.4 mg·eq·h/L, close to the value in blood. Meanwhile, the $t_{1/2}$ of [^{14}C]sorafenib in skin was highest overall among the tissues examined, and seven times higher than that in blood. These results suggest that sorafenib and its metabolites, including sorafenib *N*-oxide, accumulate in rat skin. In histopathological studies of skin toxicity associated with sorafenib in humans, the most relevant findings included keratinocyte vacuolar degeneration, the presence of intracytoplasmic eosinophilic bodies, and intraepidermal blisters in the stratum malpighii (Yang et al. 2008). Sorafenib is a multi-kinase inhibitor initially developed to inhibit the Raf1 kinase pathway (Smith et al. 2001; Lowinger et al. 2002). In addition to inhibiting tumor-cell proliferation by targeting the Raf/MEK/ERK signaling pathway, sorafenib also inhibits angiogenesis by targeting tyrosine kinases such as vascular-endothelial growth factor receptor (VEGFR-2 and VEGFR-3), platelet-derived growth factor receptor (PDGFR), Fms-like tyrosine kinase (FLT)-3, and c-KIT (Gollob 2005; Carlomagno et al. 2006; Liu et al. 2006; Wilhelm et al. 2006). Sorafenib also induces apoptosis in tumor cells via signal transducer and activator of transcription (STAT)-3 (Chen et al. 2010). Some reports have shown that the PDGF-PDGFβ receptor signal is a major proliferative and migratory stimulus for connective tissue cells during the initiation of skin-repair processes in human keratinocytes (Ansel et al. 1993; Rollman et al. 2003). According to the data from the interview form, sorafenib *N*-oxide inhibits PDGFR-β kinase four times more strongly than sorafenib (IC_{50}: 14 vs. 57 nM).

Further, Rolny et al. (2006) reported that PDGFR-β signal plays an important role in early hematopoietic development, suggesting that a decline in the PDGFR-β signal may explain the lower neutrophil counts. Combined with difference in $t_{1/2}$ values of sorafenib and sorafenib *N*-oxide between patients with both neutropenia and skin disorder and those with skin disorders alone, these data indicate that accumulation of sorafenib *N*-oxide, as well as sorafenib, causes severe neutropenia. Taken together, these data imply that accumulation of sorafenib *N*-oxide is associated with cutaneous skin toxicity or neutropenia via PDGFβ kinase inhibition, which is a reason for selecting $AUC_{N\text{-oxide}}$ and AUC ratio as predictive factors for sorafenib-induced toxicity at early stages after the first administration.

In the following, we further discuss the factors associated with risk of accumulating sorafenib *N*-oxide. In one patient with underlying intrahepatic bile duct dilatation (No. 9), the ALP value was increased by 802 U/L on day 6 after sorafenib administration; consequently, sorafenib administration was stopped at that time. Bile duct obstruction is suspected to cause accumulation of sorafenib, a substrate for CYP3A4. Two patients (No. 11 and No. 24) who received combined long-term administration of predonine also had high values of $AUC_{N\text{-oxide}}$ and AUC ratio, possibly because predonine is an inducer of the CYP3A4 protein (Usui et al. 2003; Noda et al. 2013). When patient No.11 was restarted at 400 mg/day of sorafenib (200 mg b.i.d) after suspension of treatment, $AUC_{N\text{-oxide}}$ and AUC ratio were 1.6 μg·day/mL and 0.21, respectively. Fifty days later, when no adverse effects had been observed in the patient, the attending doctor increased the dose to 600 mg (400 mg after breakfast and 200 mg after dinner), and treatment was subsequently maintained for 320 days. Based on these cases, it is possible that progression of primary diseases such as hepatitis and cirrhosis, the location of hepatocellular carcinoma, or concomitant administration of other drugs can influence the pharmacokinetic profile of sorafenib and sorafenib *N*-oxide, leading to adverse effects that ultimately result in dosage reduction/withdrawal. Patients taking sorafenib for a long period complained of diarrhea or intense lassitude. There are no detailed data available regarding the association of sorafenib or its *N*-oxide with long-term toxicity. Collectively, our result show that controlling the values of $AUC_{N\text{-oxide}}$ (≤ 2.0 μg·day/mL) and AUC ratio (≤ 0.13) makes it possible to prevent serious

adverse effects at the early stage and continue long-term therapy.

This result should be confirmed in a future study involving a larger number of patients.

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Conflict of Interest

The authors declare no conflict of interest.

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