Original Article

Association of axitinib plasma exposure and genetic polymorphisms of ABC transporters with axitinib-induced toxicities in patients with renal cell carcinoma

Hiroshi Kato[1, 3]*, Naoto Sassa[2], Masayuki Miyazaki[1, 3], Mio Takeuchi[3], Miho Asai[3], Akane Iwai[3], Yukihiro Noda[1, 3], Momokazu Gotoh[2] and Kiyofumi Yamada[1]

1 Department of Neuropsychopharmacology and Hospital Pharmacy, Nagoya University Graduate School of Medicine, Nagoya, Japan

2 Department of Urology, Nagoya University Graduate School of Medicine, Nagoya, Japan

3 Division of Clinical Sciences and Neuropsychopharmacology, Meijo University Faculty of Pharmacy, Nagoya, Japan

*Correspondence: Hiroshi Kato,

Department of Neuropsychopharmacology and Hospital Pharmacy, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi, 466-8560, Japan.

Tel: +81-52-744-2681 Fax: +81-52-744-2685

E-mail: hk-hiroshi@med.nagoya-u.ac.jp

Abstract

Purpose: Axitinib is a selective tyrosine kinase inhibitor of VEGF receptors, approved for advanced renal cell carcinoma (RCC). Associations between axitinib plasma exposure, genetic polymorphisms of ABC transporters and axitinib-induced toxicities have not been adequately explored.

Methods: Twenty RCC patients treated with axitinib were enrolled in this study. Blood samples were collected 0, 0.5, 1, 2, 4, and 6 hr after administration of axitinib on day 1 and at steady state. Plasma concentrations of axitinib were analyzed by UPLC-MS/MS. The *ABCG2* (421C>A) and *ABCB1* (1236C>T, 2677G>T/A, 3435C>T) genetic polymorphisms were determined by real-time PCR.

Results: *ABCB1* haplotype was associated with increased dose-adjusted area under the plasma concentration-time curve (AUC) of axitinib at steady state. The incidence of fatigue during therapy was associated with high $AUC_{0\text{-}6}$ of axitinib (P = 0.013). The treatment period without discontinuation or dose reduction due to adverse events in patients with high $AUC_{0\text{-}6}$ of axitinib was significantly shorter than for those with low $AUC_{0\text{-}6}$ (P = 0.024). No significant differences were found in the frequency of adverse events among the *ABCG2* genotype and *ABCB1* haplotype groups.

Conclusions: Our results have demonstrated that adverse events leading to discontinuation or dose reduction of axitinib were associated with increased axitinib plasma exposure, but not directly with genetic polymorphisms of ABC transporters. Therefore, measurement of steady state axitinib plasma concentrations may be useful in avoiding adverse events in axitinib therapy.

Keywords: axitinib, renal cell carcinoma, pharmacokinetics, adverse events, ABCG2, ABCB1

Introduction

Axitinib is an oral, selective tyrosine kinase inhibitor (TKI) of vascular endothelial growth factor (VEGF) receptors 1, 2, and 3 [1], approved for second-line treatment of advanced renal cell carcinoma (RCC). The starting dose of axitinib is 5 mg twice-daily (b.i.d.), and dose titration (to a maximum of 10 mg b.i.d.) can be performed for as long as patients tolerate the drug. A retrospective analysis using 17 trials including pharmacokinetic data has shown that the area under the plasma concentration-time curve (AUC) of axitinib and diastolic blood pressure correlate with progression-free and overall survival in RCC patients [2].

Noda et al. reported that RCC patients with excessive plasma trough concentrations of sunitinib (a multitargeted TKI used mainly in first line treatment of advanced RCC) together with its active metabolite SU12662, had a greater incidence of grade ≧3 adverse events and worse clinical outcomes. These authors suggested the importance of measurement of plasma concentrations of sunitinib and SU12662 in order to avoid early discontinuation of sunitinib treatment [3].

Breast cancer resistance protein (BCRP, gene code *ABCG2*) and P-glycoprotein (MDR1, gene code *ABCB1*) are efflux adenosine triphosphate binding cassette (ABC) transporters expressed on the apical membranes of enterocytes, and are involved in the absorption and excretion of various drugs [4]. The genetic polymorphisms of *ABCG2* 421C>A and *ABCB1* 1236C>T, 2677G>T/A, and 3435C>T are known to cause decreased protein expression [5, 6]. Several studies have demonstrated that *ABCG2* and *ABCB1* polymorphisms affect plasma concentrations of oral anticancer drugs. For example, plasma concentration of sunitinib was reported to be remarkably higher in a patient with the *ABCG2* 421A/A genotype (homo variant) than in wild type patients [7]. In addition, Hamada et al. reported that non-small cell lung cancer patients with all T alleles in the *ABCB1* haplotype (1236C>T,

2677G>T/A, 3435C>T) had higher plasma concentrations of erlotinib, a potent TKI of epidermal growth factor receptor (EGFR), than those with other haplotypes [8].

Individual differences in axitinib pharmacokinetics among patients with genetic polymorphisms of ABC transporters have not yet been clarified. Associations between axitinib plasma exposure, genetic polymorphisms of ABC transporters and axitinib-induced toxicities have not been adequately explored. Therefore, in the present study, we examined the effects of genetic polymorphisms in *ABCG2* and *ABCB1* on plasma concentrations of axitinib in RCC patients. We also evaluated an association of plasma concentrations of axitinib with adverse events and clinical efficacy.

Patients and Methods

Patients

This study was an exploratory retrospective observational study. Twenty Japanese RCC patients treated with axitinib at Nagoya University Hospital were enrolled between January 2013 and November 2015. Dose-up or dose reduction of axitinib was conducted based on clinical efficacy and toxicity. This study was approved by the ethical committee of Nagoya University Hospital. Informed consent was obtained from all participants included in this study.

Mesurement of plasma concentration of axitinib

Blood samples were collected 0, 0.5, 1, 2, 4, and 6 hr after administration of axitinib (6 points) on day 1 and at steady state. Steady state was defined as on and after day 5. Plasma was separated by

centrifugation of blood samples and stored at -30°C prior to analysis. Plasma concentrations of axitinib were analyzed by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). The area under the plasma concentration-time curve from 0 to 6 hr (AUC_{0-6}) of axitinib was calculated by the trapezoidal method.

UPLC-MS/MS analysis was performed using the ACQUITY UPLC® System (Waters, MA, USA). Analytes were separated on an ACQUITY UPLC® BEH C_{18} column (1.7 μm, 50 mm × 2.1 mm) and column temperature was maintained at 40°C. The mobile phase consisted of (A) 0.1% formic acid and (B) methanol at a flow rate of 0.1 mL/min. Gradient conditions were from 25% to 95% mobile phase B for 4 min, held for 2 min, and then ramped from 95% to 25% mobile phase B (total run time of 8 min). Tandem mass spectrometry (MS/MS) was performed in the positive ion electrospray ionization mode. Multiple reaction transitions were set at *m/z* 387.1 to *m/z* 356.1 for axitinib, and *m/z* 394.2 to *m/z* 278.1 for erlotinib as an internal standard. Cone voltages were 45 and 54 V for axitinib and erlotinib, respectively. Collision energies were 20 and 32 eV for axitinib and erlotinib, respectively.

Intra- and inter-day precisions were less than 15% of the coefficient of variation, and the intra- and inter-day accuracies (% bias) were within ±15% in our method.

Genotyping

Genotyping was performed for the following single-nucleotide polymorphisms (SNPs): *ABCG2* 421C>A (rs2231142), *ABCB1* 1236C>T (rs1128503), *ABCB1* 2677G>T/A (rs2032582), *ABCB1* 3435C>T (rs1045642). Genomic DNA was prepared from blood clots using the QIAamp

DNA Blood Mini Kit (QIAGEN, CA, USA). Purified genomic DNA was stored at -30°C prior to genotyping. Allelic variations were determined using the TaqMan® SNP genotyping assay (Applied Biosystems, CA, USA). Gene fragments were amplified by polymerase chain reaction (PCR) using StepOne™ Real-Time PCR System (Applied Biosystems). Amplification conditions consisted of an initial denaturation step at 95°C for 10 min, followed by 50 cycles of denaturation at 92°C for 15 sec, and annealing and extension at 58°C for 1 min.

Assessment of safety and efficacy

We retrospectively evaluated adverse events using medical record. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0). We defined time to treatment failure (TTF) as the period from the day axitinib treatment was started until discontinuation of axitinib treatment for any reason.

Statistical analysis

Correlation of *ABCB1* haplotype with dose-adjusted AUC_{0-6} of axitinib was evaluated by Mann-Whitney *U* test. Correlation of axitinib AUC_{0-6} with axitinib-induced adverse events was also evaluated by Mann-Whitney *U* test. The Kaplan-Meier method was used to plot time-to-event curves and statistical significance was estimated by log-rank test. All statistical analyses were performed using SPSS® Statistics version 22 (IBM, NY, USA). *P* values < 0.05 were considered to denote statistically significant differences.

Results

Patient characteristics

Patient characteristics are shown in Table 1. Nineteen patients were administered axitinib 5 mg b.i.d. as starting dose, while one patient received 4 mg b.i.d. (also as starting dose). Dose escalation to 7 mg b.i.d. was conducted in 6 patients. Four patients were administrated axitinib as pre-surgical therapy, which was approved by the Institutional Review Board. Blood samples were collected on day 1 (not collected: n=1) and at steady state (day 5; n=1, day 8; n=15, day 9; n=1, day 29; n=1, not collected: n=2).

Relationship between genetic polymorphisms and plasma concentrations of axitinib

At the standard dose (5 mg b.i.d.), the $AUC_{0\text{-}6}$ and maximum concentration (C_{max}) of axitinb in one patient carrying *ABCG2* 421A/A (homozygous variant) genotype (70.1 ng/mL) were markedly higher than in those carrying *ABCG2* 421C/A (heterozygous variant; 22.9 ng/mL, median) or C/C (wild type; 25.4 ng/mL, median) genotype at steady state (Table 2). The presence of T/T(A)/T in the *ABCB1* (1236C>T, 2677G>T/A, 3435C>T) haplotype was associated with a significant increase in dose-adjusted $AUC_{0\text{-}6}$ of axitinib at steady state ($P = 0.043$, Fig. 1).

Relationship between plasma concentrations of axitinib and adverse events

We next assessed whether there was an association of axitinib plasma exposure with axitinib-induced adverse events during treatment with the starting dose (Table 3). The incidence of fatigue (grade $\geqq 1$) during axitinib therapy was significantly associated with the high steady state

AUC_{0-6} of axitinib (P = 0.013). On the other hand, there were no significant correlations of the incidence of hoarseness (grade ≧1), diarrhea (grade ≧1) or hypertension (grade ≧2) with the steady state AUC_{0-6} of axitinib.

Patients were divided into two groups according to the median (97.3 ng h/mL) steady state AUC_{0-6} of axitinib: high AUC (≧ median) group (n=9) and low AUC (< median) group (n=9). Patients' characteristics (age, gender, initial axitinib dose, and baseline laboratory data) were not significant difference between both groups. Seven of 9 patients (77.8%) in the high AUC group, but only 2 of 9 patients (22.2%) in the low AUC group underwent discontinuation or dose reduction of axitinib therapy due to adverse events. Fig. 2a shows the Kaplan-Meier plot of time to discontinuation or dose reduction of axitinib therapy due to adverse events. The reasons of censoring were progression disease and pre-surgical case in the high AUC group, and then progression disease, pre-surgical case, changing hospital, and under continuation of axitinib therapy in the low AUC group. The treatment period without discontinuation or dose reduction due to adverse events in the high AUC group was significantly shorter than that in the low AUC group (median: 35 days versus not reached, P = 0.024).

Relationship between genetic polymorphisms and adverse events

We also evaluated an association of genetic polymorphisms of *ABCG2* and *ABCB1* with axitinib-induced adverse events (Table 4). No significant differences were found in the frequency of adverse events or discontinuation/dose reduction induced by adverse events among the *ABCG2* 421C>A genotype and *ABCB1* (1236C>T, 2677G>T/A, 3435C>T) haplotype groups.

Association of TTF with hypertension or plasma concentrations of axitinib

Four patients administrated axitinib as pre-surgical therapy were excluded from the evaluation of TTF. Median TTF of axitinib therapy was 122 days. TTF in patients with grade ≧2 hypertension (n=7) was significantly longer than in those with grade 0-1 hypertension (n=9) (median: 267 versus 52 days, $P = 0.017$, Fig. 2b). However, no significant difference in TTF was observed between high and low AUC groups (median: 122 versus 260 days, $P = 0.924$).

Discussion

It has been previously demonstrated that axitinib is a substrate for BCRP and MDR1 in *in vivo* and *in vitro* analyses [9]. In the present study, plasma concentrations of axitinb in patients carrying the *ABCG2* 421A/A genotype or *ABCB1* (1236C>T, 2677G>T/A, 3435C>T) T/T(A)/T haplotype were increased at steady state. The reduction of BCRP and MDR1 protein levels induced by genetic polymorphisms may have contributed to high exposure to axitinib. Nevertheless, Brennan et al. suggested that genotype-based adjustment of axitinib dose in individual patients is not warranted because no statistically significant associations between genetic polymorphisms and axitinib plasma exposure were observed in meta-analysis using data pooled from clinical pharmacology trials in healthy volunteers [10]. However, pharmacokinetic data were utilized based on single-dosing of axitinib, and the effect of gene polymorphisms of ABC transporters was evaluated only in *ABCB1* (2677G>T/A, 3435C>T) but not *ABCG2* in that analysis. Accordingly, it is difficult to clarify the influence of genetic polymorphisms of ABC transporters on axitinib pharmacokinetics because

mechanisms underlying individual differences in plasma concentrations of oral molecular targeting agents are more complex, including other factors such as metabolic enzymes and co-administrated drugs. Further pharmacogenetic and pharmacokinetic investigations are needed to apply these data to clinical settings.

In the randomized phase 3 trial (AXIS study), the most frequent adverse events associated with axitinb therapy were diarrhea, hypertension, and fatigue (55%, 40%, and 39%, respectively). Furthermore, the most common adverse event leading to discontinuation was fatigue (4 of 14, 29%), subsequently transient ischemic attack (3 of 14, 21%) in the axitinib arm [11]. Our study also indicated that the most common adverse event leading to discontinuation and dose reduction was fatigue (4 of 9, 44%), subsequently diarrhea (2 of 9, 22%). Further investigations into factors predictive of adverse events leading to discontinuation and dose reduction are required in order to maintain axitinib therapy.

Rini et al. showed that patients who achieved 1- to 2- hour post-first-dose (on day 1) axitinib plasma concentrations (1 point) within a specific range (quartile 3 of 4) had the best clinical outcome in a retrospective study [12]. They also described that patients with the highest axitinib plasma concentrations (quartile 4) had the highest incidence of severe adverse events leading to early dose reduction or treatment discontinuation, which result in suboptimal efficacy. It is necessary to measure plasma concentrations and regulate axitinib dose in order to avoid adverse events.

When 5 mg axitinib was administered alone, median time to maximal plasma concentration (T_{max}) was 2.5 hr (range 1.5-4.0 hr) and mean C_{max} was 33.5 ng/mL (range 10.1-64.1 ng/mL) for healthy volunteers in a phase 1 trial [13]. In the present study, median T_{max} was 4.0 hr (range 2.0-6.0

hr) and mean C_{max} was 22.8 ng/mL (range 2.2-58.1 ng/mL) on day 1. Some patients might have gastrointestinal hypomotility and delayed absorption according to T_{max} values. Furthermore, the AUC_{0-6} on day 1 was not correlated with those at steady state (coefficient of determination, r^2 = 0.173). It is indicated that the AUC_{0-6} on day 1 was not useful for alternate of those at steady state.

Previous studies have demonstrated a correlation between high AUC of some TKIs (imatinib, nilotinib, sunitinib, and erlotinib) and increased toxicities [7, 14-17]. Regarding gefitinib, it has been reported that adverse events are related to plasma exposure (AUC) to gefitinib but not genetic polymorphisms of its metabolizing enzymes and transporters [18]. Therefore, it is suggested that measurement of plasma concentrations after beginning gefitinib therapy, rather than analysis of genetic polymorphisms before initiating therapy, could be beneficial. In the present study, we showed that high AUC of axitinib was associated with discontinuation and dose reduction due to adverse events, whereas genetic polymorphism was not associated with adverse events of axitinib therapy. Thus, our results are in agreement with previous reports regarding other molecular targeting agents. However, measurement of AUC in clinical settings is complicated because a large amount of blood sampling is required. Therefore, a simple monitoring method would be preferred. In previous studies with sunitinib, it was reported that trough concentration and AUC of this agent were highly correlated [19], and monitoring of trough concentration of sunitinib was useful for avoiding severe toxicities [3]. We found in the present study that axitinib trough concentration was not correlated with the AUC at steady state (r^2 = 0.084). Establishment of an alternative monitoring method for axitinib exposure is required.

Regarding clinical efficacy of axitinib, longer TTF was associated with the incidence of grade

≧2 hypertension in our data. Hypertension is a common adverse event induced by agents inhibiting the VEGF pathway [20]. In a previous retrospective analysis, sunititib-induced hypertension was associated with improved survival in patients with metastatic RCC [21]. Additionally, axitinib-induced hypertension was also associated with improved survival for RCC in updated results from the AXIS study [22] and for other solid tumors [23]. Hypertension, which is a class adverse effect, may be useful as a biomarker of clinical efficacy for VEGF inhibitors.

In a randomized, double-blind phase 2 trial in patients with previously untreated metastatic RCC, AUC of axitinib and progression-free survival were not strongly correlated, and the correlation between AUC of axitinib and blood pressure was weak [24]. Our results showed that axitinib exposure was not related to TTF and no correlation was observed between AUC of axitinib and hypertension. Accordingly, axitinib plasma exposure cannot be a predictor of clinical efficacy. Nevertheless, measurements of axitinib plasma concentrations may be useful as an indicator of adverse events. However, a specific plasma concentration range related to adverse events induced by axitinib cannot be defined in the present exploratory study, with its single-center design and small sample size. Further clinical pharmacokinetic and pharmacodynamic studies are necessary to determine the threshold axitinib plasma concentration required to avoid adverse events in axitinib therapy.

In conclusion, our results have demonstrated that adverse events leading to discontinuation or dose reduction of axitinib were associated with increased axitinib plasma exposure, but not directly with genetic polymorphisms of ABC transporters. Therefore, measurement of axitinib plasma concentrations at steady state may be useful for avoidance of adverse events in axitinib therapy. On

the other hand, monitoring of hypertension may be benefical in predicting clinical efficacy, rather than measurement of axitinib plasma concentrations. Further prospective studies with a larger sample size are needed to establish predictive indicators of toxicity and clinical efficacy of axitinib therapy.

Acknowledgments

This work was supported in part by JSPS KAKENHI Grant Number JP 16H00497.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Hu-Lowe DD, Zou HY, Grazzini ML et al (2008) Nonclinical antiangiogenesis and antitumor activities of axitinib (AG-013736), an oral, potent, and selective inhibitor of vascular endothelial growth factor receptor tyrosine kinases 1, 2, 3. Clin Cancer Res 14:7272-7283
2. Rini BI, Garrett M, Poland B et al (2013) Axitinib in metastatic renal cell carcinoma: results of a pharmacokinetic and pharmacodynamic analysis. J Clin Pharmacol 53:491-504
3. Noda S, Otsuji T, Baba M et al (2015) Assessment of sunitinib-induced toxicities and clinical outcomes based on therapeutic drug monitoring of sunitinib for patients with renal cell carcinoma. Clin Genitourin Cancer 13:350-358
4. Terada T, Hira D (2015) Intestinal and hepatic drug transporters: pharmacokinetic, pathophysiological, and pharmacogenetic roles. J Gastroenterol 50:508-519
5. Imai Y, Nakane M, Kage K et al (2002) C421A polymorphism in the human breast cancer resistance protein gene is associated with low expression of Q141K protein and low-level drug resistance. Mol Cancer Ther 1:611-616.
6. Kimchi-Sarfaty C, Oh JM, Kim IW et al (2007) A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science 315:525-528
7. Mizuno T, Fukudo M, Terada T et al (2012) Impact of genetic variation in breast cancer resistance protein (BCRP/ABCG2) on sunitinib pharmacokinetics. Drug Metab Pahrmacokinet 27:631-639
8. Hamada A, Sasaki J, Saeki S et al (2012) Association of ABCB1 polymorphisms with erlotinib pharmacokinetics and toxicity in Japanese patients with non-small-cell lung cancer.

Pharmacogenomics 13:615-624

9. Poller B, Iusuf D, Sparidans RW et al (2011) Differential impact of P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2) on axitinib brain accumulation and oral plasma pharmacokinetics. Drug Metab Dispos 39:729-735
10. Brennan M, Williams JA, Chen Y et al (2012) Meta-analysis of contribution of genetic polymorphisms in drug-metabolizing enzymes or transporters to axitinib pharmacokinetics. Eur J Clin Pharmacol 68:645-655
11. Rini BI, Escudier B, Tomczak P et al (2011) Comparative effectiveness of axitinib versus sorafenib in advanced renal cell carcinoma (AXIS): a randomised phase 3 trial. Lancet 378:1931-1939
12. Rini BI, de La Motte Rouge T, Harzstark AL et al (2013) Five-year survival in patients with cytokine-refractory metastatic renal cell carcinoma treated with axitinib. Clin Genitourin Cancer 11:107-114
13. Chen Y, Jiang J, Zhang J et al (2011) A Phase I study to evaluate the pharmacokinetics of axitinib (AG-13736) in healthy Chinese volunteers. Int J Clin Pharmacol Ther 49:679-687
14. Delbaldo C, Chatelut E, Ré M et al (2006) Pharmacokinetic-pharmacodynamic relationships of imatinib and its main metabolite in patients with advanced gastrointestinal stromal tumors. Clin Cancer Res 12:6073-6078
15. Larson RA, Yin OQ, Hochhaus A et al (2012) Population pharmacokinetic and exposure-response analysis of nilotinib in patients with newly diagnosed Ph+ chronic myeloid leukemia in chronic phase. Eur J Clin Pharmacol 68:723-733

16. Houk BE, Bello CL, Poland B et al (2010) Relationship between exposure to sunitinib and efficacy and tolerability endpoints in patients with cancer: results of a pharmacokinetic/pharmacodynamic meta-analysis. Cancer Chemother Pharmacol 66:357-371
17. Fukudo M, Ikemi Y, Togashi Y et al (2013) Population pharmacokinetics/pharmacodynamics of erlotinib and pharmacogenomic analysis of plasma and cerebrospinal fluid drug concentrations in Japanese patients with non-small cell lung cancer. Clin Pharmacokinet 52:593-609
18. Kobayashi H, Sato K, Niioka T et al (2015) Relationship among gefitinib exposure, polymorphisms of its metabolizing enzymes and transporters, and side effects in Japanese patients with non-small-cell lung cancer. Clin Lung Cancer 16:274-281
19. de Wit D, Gelderblom H, Sparreboom A et al (2014) Midazolam as a phenotyping probe to predict sunitinib exposure in patients with cancer. Cancer Chemother Pharmacol 73:87-96
20. Roodhart JM, Langenberg MH, Witteveen E et al (2008) The molecular basis of class side effects due to treatment with inhibitors of the VEGF/VEGFR pathway. Curr Clin Pharmacol 3:132-143
21. Rini BI, Cohen DP, Lu DR et al (2011) Hypertension as a biomarker of efficacy in patients with metastatic renal cell carcinoma treated with sunitinib. J Natl Cancer Inst 103:763-773
22. Motzer RJ, Escudier B, Tomczak P et al (2013) Axitinib versus sorafenib as second-line treatment for advanced renal cell carcinoma: overall survival analysis and updated results from a randomised phase 3 trial. Lancet Oncol 14:552-562
23. Rini BI, Schiller JH, Fruehauf JP et al (2011) Diastolic blood pressure as a biomarker of axitinib efficacy in solid tumors. Clin Cancer Res 17:3841-3849.
24. Rini BI, Melichar B, Fishman MN et al (2015) Axitinib dose titration: analyses of exposure,

blood pressure and clinical response from a randomized phase II study in metastatic renal cell carcinoma. Ann Oncol 26:1372-1377

Figure legends

Figure 1. Association between *ABCB1* haplotype (1236C>T, 2677G>T/A, 3435C>T) and dose-adjusted AUC_{0-6} of axitinib on day1 (a), and at steady state (b). Box plots represent the median and 25th and 75th percentiles. Whiskers represent ranges. Statistical significance was evaluated by Mann-Whitney *U* test. N.S. not significant.

Figure 2. (a) Kaplan-Meier plot of time to discontinuation or dose reduction of axitinib therapy due to adverse events in patients with high (≧ median, n=9) versus low (< median, n=9) steady state AUC_{0-6} of axitinib. Median value: 97.3 ng h/mL. (b) Kaplan-Meier plot of time to treatment failure (TTF) in patients with grade ≧2 (n=7) versus grade 0-1 (n=9) hypertension. Hypertension was graded according to the CTCAE v4.0. *P* values based on log-rank test.

Tables

Table 1. Patient Characteristics

Characteristics	(n=20)
Median age (range), years	67 (35-78)
Gender, n (%)	
Male	14 (70.0)
Female	6 (30.0)
Median weight (range), kg	55.9 (35.5-88.0)
Histopathology of RCC [a]	
Clear cell RCC	20
Others	0
Nuclear grade	
Grade 1	0
Grade 2	2
Grade 3	13
Unknown	5
cTNM classification (AJCC [b])	
cT1	5
cT2	0
cT3	11
cT4	4
cN-	15
cN+	5
cM-	10
cM+	10

The number of regimens with molecular targeted agents before use of axitinib	
None (with presurgical)	4
1	13
≧2	3
MSKCC [c] risk classification	
Favorable	4
Intermediate	11
Poor	5
Median laboratory data (range)	
AST, IU/L	24 (12-55)
ALT, IU/L	19 (7-54)
BUN, mg/dL	18 (12-32)
Creatinine, mg/dL	0.89 (0.45-1.99)
Initial dose, n (%)	
10mg/day	19 (95.0)
8mg/day	1 (5.0)

[a] RCC: Renal Cell Carcinoma

[b] AJCC: American Joint Committee on Cancer

[c] MSKCC: Memorial Sloan-Kettering Cancer Center

Table 2. Influence of genetic polymorphism in *ABCG2* 421C>A on axitinib plasma concentrations at the standard dose

ABCG2	Day1			Steady State		
421C>A Genotype	Patient (n)	AUC_{0-6} (ng h/mL)	C_{max} (ng/mL)	Patient (n)	AUC_{0-6} (ng h/mL)	C_{max} (ng/mL)
Wild (C/C)	9	72.2 (64.4-97.1)	24.5 (19.6-26.6)	8	103.1 (82.5-123.3)	25.4 (20.5-35.5)
Hetero (C/A)	8	54.1 (30.7-75.2)	15.6 (10.0-22.6)	8	92.7 (71.8-110.4)	22.9 (13.9-27.2)
Homo (A/A)	1	93.1	29.1	1	301.6	70.1

Results are expressed as median (interquartile range)

Table 3. Relationships between steady state AUC_{0-6} of axitinib and adverse events

Adverse events [a]		Patients (n)	Steady State AUC_{0-6} (ng h/mL) [b]	*P* Value [c]
Fatigue (grade ≧1)	(+)	6	129.4 (108.4-206.3)	0.013
	(-)	12	87.1 (71.8-108.8)	
Hoarseness (grade ≧1)	(+)	5	133.3 (117.2-227.9)	0.075
	(-)	13	93.8 (74.6-105.4)	
Diarrhea (grade ≧1)	(+)	4	122.2 (100.0-181.7)	0.233
	(-)	14	97.3 (76.4-129.1)	
Hypertension (grade ≧2)	(+)	8	104.1 (80.2-148.8)	0.829
	(-)	10	97.3 (84.9-129.3)	

For two patients, blood samples were not collected at steady state

[a] Adverse events were graded according to the CTCAE v4.0

[b] Results are expressed as median (interquartile range)

[c] Mann-Whitney *U* test

Table 4. Relationship between genetic polymorphism and adverse events

Events [a], n (%)	*ABCG2* genotype [c]			*ABCB1* haplotype [d]		
	With A allele (n=9)	Without A allele (n=11)	*P* Value [e]	With T/T(A)/T (n=12)	Without T/T(A)/T (n=8)	*P* Value [e]
Fatigue (≧Grade1)	2 (22.2)	4 (36.4)	0.642	4 (33.3)	2 (25.0)	1.000
Hoarseness (≧Grade1)	2 (22.2)	4 (36.4)	0.642	3 (25.0)	3 (37.5)	0.642
Diarrhea (≧Grade1)	3 (33.3)	2 (18.1)	0.617	4 (33.3)	1 (12.5)	0.603
Hypertension (≧Grade2)	4 (44.4)	5 (45.5)	1.000	6 (50.0)	3 (37.5)	0.670
Discontinuation/ Dose reduction [b]	3 (33.3)	6 (54.5)	0.406	6 (50.0)	3 (37.5)	0.670

[a] Adverse events were graded according to the CTCAE v4.0

[b] Discontinuation or dose reduction due to adverse events

[c] *ABCG2*: 421C>A [d] *ABCB1*: 1236C>T, 2677G>T/A, 3435C>T

[e] Fisher's exact test

Fig. 1

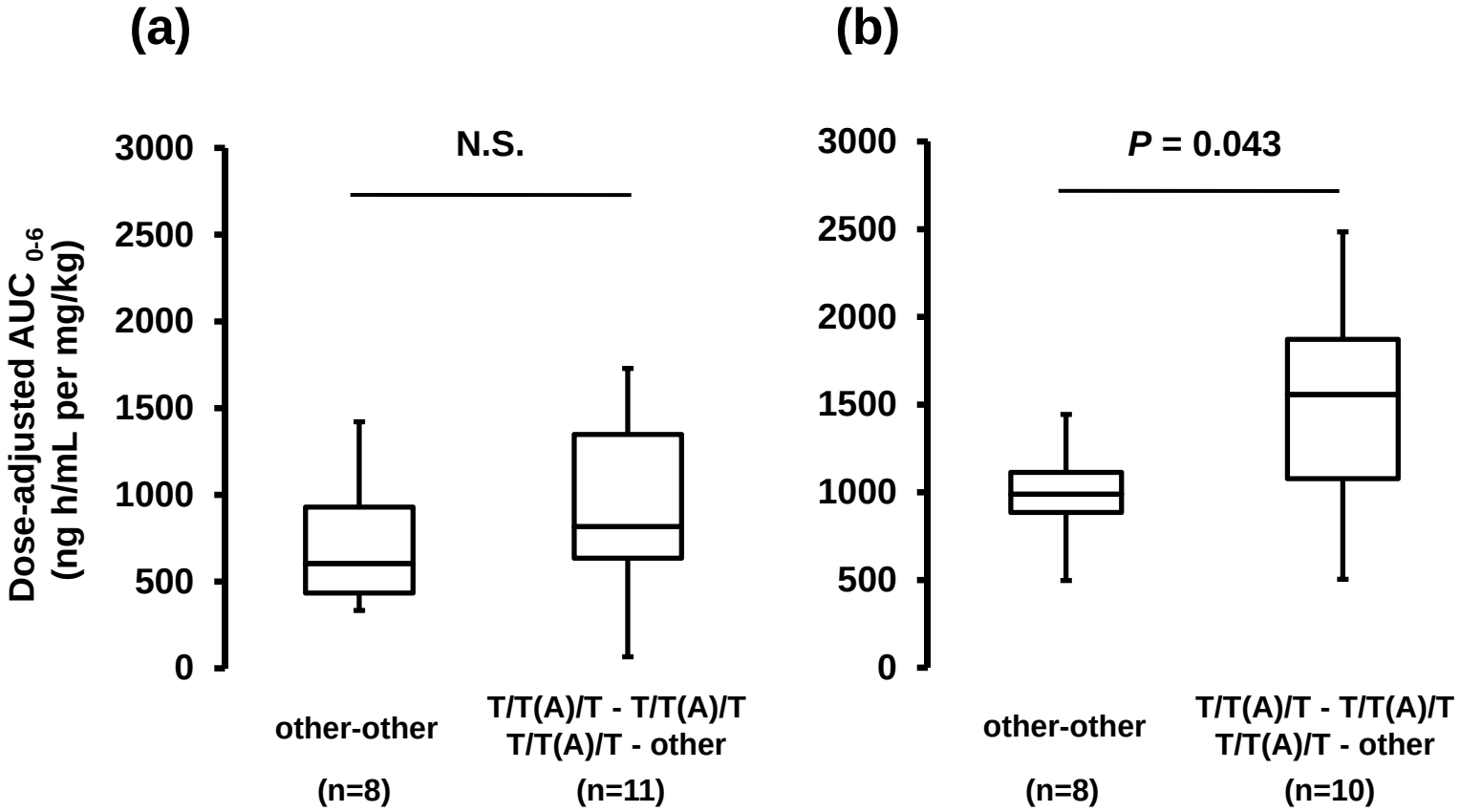
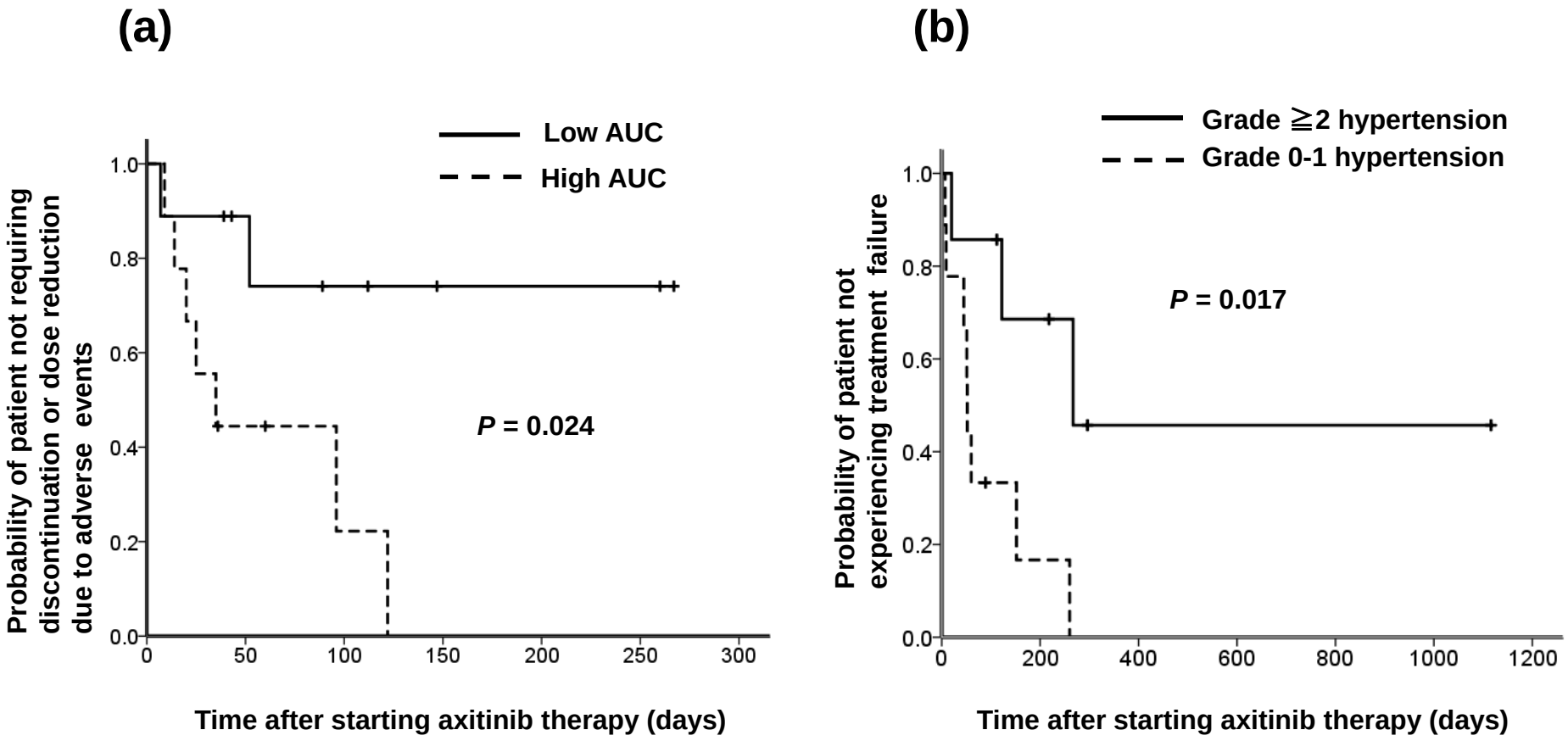

(a)
(b)
N.S.
P = 0.043
Dose-adjusted AUC 0-6
(ng h/mL per mg/kg)
3000
2500
2000
1500
1000
500
0
other-other
(n=8)
T/T(A)/T - T/T(A)/T
T/T(A)/T - other
(n=11)
other-other
(n=8)
T/T(A)/T - T/T(A)/T
T/T(A)/T - other
(n=10)
ABCB1（1236C>T, 2677G>T/A, 3435C>T）haplotype

Fig. 2


(a)
Low AUC
High AUC
Probability of patient not requiring discontinuation or dose reduction due to adverse events
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
250
300
P = 0.024
Time after starting axitinib therapy (days)
(b)
Grade ≧2 hypertension
Grade 0-1 hypertension
Probability of patient not experiencing treatment failure
1.0
0.8
0.6
0.4
0.2
0.0
0
200
400
600
800
1000
1200
P = 0.017
Time after starting axitinib therapy (days)