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Quantitative Measurements of Sorafenib in Patients with Advanced Hepatocellular Carcinoma

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ABSTRACT

***Aim:** A kinase inhibitor, sorafenib, has been approved for the treatment of advanced hepatocellular carcinoma (HCC). In this study, we aimed to determine the concentration of sorafenib in patients with HCC who were administered sorafenib (n = 37),and investigated the relationship between oral dosage and serum concentrations of sorafenib.*

***Methods:** After precipitating proteins from serum with acetonitrile along with an internal control, samples were evaporated to dryness using a rotary evaporator. Then, residues were dissolved in the mobile phase of high-performance liquid chromatography (HPLC). Quantitative measurements of sorafenib were performed using reverse-phase HPLC with ultraviolet detection with isocratic elution.*

***Results:** The average steady-state sorafenib concentration was 4.1± 1.7 mg/L (800 mg/day, maintenance dose), 6.1±3.9 mg/L (400 mg/day), or 4.5±4.5 mg/L (200 mg/day) in patients with HCC who were continuously administered sorafenib orally. No significant correlation was detected between the serum concentration and the maintenance dose of sorafenib.*

***Conclusions:** We quantitatively determined the concentration of sorafenib in sera obtained from patients with HCC. The lack of a correlation between serum levels of sorafenib and the orally administered dose in patients suggests that it may be critical to maintain an effective drug concentration in plasma for a long period of time.*

Keywords: *hepatocellular carcinoma, high-performance liquid chromatography, quantitative measurements, sorafenib*

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most malignant types of tumor and one of the leading causes of cancer-related deaths. Sorafenib is a first-line oral drug for advanced-stage HCC (aHCC) [1]. It is a multi-kinase inhibitor, in that it inhibits several serine/threonine kinases, including the c-Raf and b-Raf tyrosine kinases; c-Kit; Fms-like tyrosine kinase 3 (Flt-3); rearranged during transfection (Ret); vascular endothelial growth factor receptor (VEGFR)-1, -2, and -3; and platelet-derived growth factor receptor (PDFGR) -β [2–4] Sorafenib may inhibit the Raf/MEK/ERK cascade in tumor cells, resulting in the suppression of tumor growth [3-5].

Furthermore, sorafenib can also suppress VEGF-2/3 and PDGFR-β signals in tumor-associated endothelial cells, thereby blocking angiogenesis. Other reported effects include suppression of tumor vessel growth [6], improved hypoxia conditions [7], and

sensitization of cells to apoptosis [8]. Several studies have established that sorafenib prolongs the survival of patients with aHCC via these synergistic activities.

Combination treatments including sorafenib and other therapies for HCC have been the subject of recent attention [9].The initial and maintenance doses of sorafenib have received increased attention in recent times in the field of HCC chemotherapy, because a proper dose for each patient is necessary to obtain the maximal effect of sorafenib. To properly administer sorafenib, it is important to assess whether the plasma concentration of sorafenib reaches effective ranges in patients with HCC.

Thus, in the present study, we measured sorafenib concentrations in patients with aHCC. Furthermore, we studied whether the oral dose correlated with the serum concentration of sorafenib.

Materials and Methods

Materials

Itraconazole was purchased from WAKO (Tokyo, Japan). Sorafenib (BAY 43-9006) was purchased from ChemScene (Monmouth Junction, NJ, USA).

Patients

A total of 37 adult Japanese patients with HCC who provided written consent at the Toho University Omori Hospital Critical Care Center were admitted to Omori Hospital between 2002 and 2014. Blood samples were collected at 8 h post-dose after continual oral administration of drug for at least 10 days.

Extraction of Sorafenib from Serum

The methods used to extract sorafenib have been described previously by Escudero–Ortiz [10] and Heinz [11]. A total of 95 µLof patient serum was mixed with 2.5 µL of itraconazole, as an internal standard for high-performance liquid chromatography (HPLC), and 350 µL of acetonitrile.

The mixture was centrifuged at 8900 × *g* for 10 min and supernatants were evaporated to dryness using a rotary evaporator (MicroVac, model MV-100; Tomy, Tokyo, Japan). The residue was resolved in 400 µL of the mobile phase of HPLC.

Reverse-Phase HPLC Conditions

A total of 40 µL of an aliquot was injected onto a Wakopak Handy ODS (4.6 i.d. x 150 mm, WAKO) and sorafenib and its metabolites were separated using isocratic elution of 20 mmol/L ammonium acetate: acetonitrile (47:53) with a mobile phase at a flow rate of 1.0 mL/min. The eluent was detected at 260 nm by using SPD-10AV (Shimadzu, Kyoto Japan) at 40°C with a column oven (Model 556, GL Science, Tokyo, Japan). The peak areas of sorafenib and itraconazole were calculated using a Model LC-8020 Multi Station (Tosoh Bioscience, Tokyo, Japan). Sorafenib concentrations were determined using an absolute standard curve method and values were normalized to those of itraconazole, an internal standard.

Standard Curve

Aliquots that contained 0–22.2 mg/L sorafenib were used for the external calibration curve methods. A standard curve was obtained from each concentration of sorafenib in 400 µL of the mobile phase for each measurement.

Statistical Analysis

All experimental data were represented as means ± standard deviation (S.D.) values and analyzed by the Kruskai–Wallis test; statistical significance was defined as a *P*-value less than 0.05.

Ethical Approval

The study protocol was designed to adhere to ethical guidelines for epidemiological studies and was approved by the research ethics committee of Toho University Omori Medical Center (approval number M1691-4). All data collected were anonymized so that it was not possible for third parties to easily identify specific individuals.

Results and Discussion

Determination of Sorafenib Levels in Human Sera

To determine the concentration of sorafenib in patients with aHCC, we used modified HPLC-UV methods based on those described in previously published studies by Escudero–Ortiz [10] and Heinz [11]. A typical chromatogram is shown in Figure 1. From an analysis of normal human serum without sorafenib and/or an internal standard, it was evident that arrowedpeak (↓) represented sorafenib derived from sera.

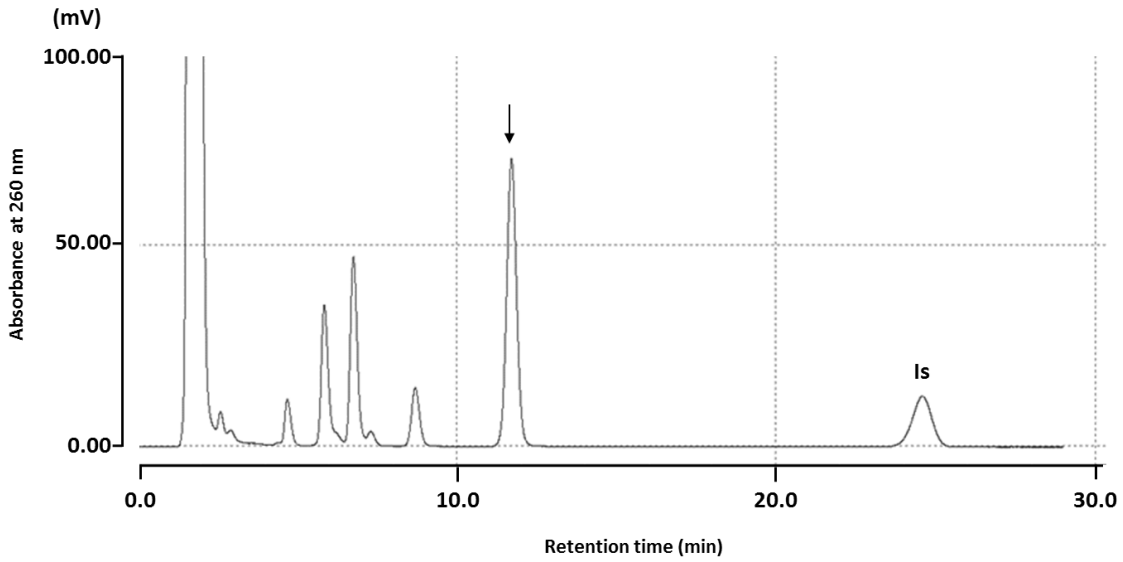

Figure1. *A typical chromatogram used to determine the presence of sorafenib. Arrowed Peaks, sorafenib; Is, internal standard.*

The linearity of our method was studied for concentrations that ranged from 0 to 22.2 mg/L sorafenib with correlation coefficients of 0.9993 (data not shown) and a detection limit for sorafenib of 0.007 mg/L (S/N = 5).

Overall, this method exhibited a robust correlation and sufficient sensitivity for determining the concentration of sorafenib in human sera.

Sorafenib Concentrations in Serum

Table 1 shows the characteristics of patients with aHCC. All patients received continuous oral administration of sorafenib for 3–4 weeks. The plasma concentration of sorafenib in patients with HCC in the steady-state was determined (Table 2). To test whether the sorafenib concentration in sera depended upon the administered dose, we studied the relationship between the dose and plasma concentration of sorafenib (Fig. 2A). Overall, the sorafenib concentration did not show a significant association with the administered dose ($p = 0.131$). Furthermore, to study whether the body weight of patients affected the concentration of sorafenib, we studied the relationship between the weight-normalized dose of patients and the sorafenib concentration. Figure 2B shows that the concentration of sorafenib showed no significant correlation with the weight-normalized dose (correlation coefficient, $R^2 = 0.0053$).

Table1. *Baseline patient characteristics.*

Characteristics	Means± S.D.	Range
Number of cases	37	
Age (years)	70.0 ± 7.2	(56-84)
Male sex (no. [%])	28[75.7]	
Weight (kg)	56.6 ± 9.6	(37.6-72.6)
Body surface area (m^2)	1.575 ± 0.150	(1.246-1.800)
Initiation dosage of sorafenib (mg/kg/day)	7.1 ± 3.4	(2.8-16.3)
Laboratory Data	Means± S.D.	Range
Ammonia (μg/dL)	52 ± 42	(15-211)
Total protein (g/dL)	7.6 ± 1.0	(5.4-10.2)
Albumin (g/dL)	3.4 ± 0.6	(2.0-4.5)
Total bilirubin (mg/dL)	1.0 ± 0.5	(0.4-2.4)
Prothrombin activity (%)	84 ± 153	(54-124)
Aspartate aminotransferase (IU/L)	78 ± 78	(15-481)
Alanine aminotransferase (IU/L)	42 ± 34	(5-175)
Lactate dehydrogenase (IU/L)	258 ± 85	(138-548)
Alkaline phosphatase (IU/L)	544 ± 507	(143-2893)
γ-Glutamyltranspeptidase (IU/L)	192 ± 222	(24-883)

Cholin esterase (IU/L)	164 ± 72	(44-297)
Blood urea nitrogen (mg/dL)	18±9	(9-48)
Creatinine (mg/dL)	0.86 ± 0.39	(0.41-2.25)
Blood sugar (mg/dL)	128 ± 34	(74-207)
C-reactive protein (mg/dL)	1.4 ± 2.3	(0.0-8.1)
White blood cell ($\times 10^3/\mu L$)	4.8 ± 2.3	(2.1-11.8)
Red blood cell ($\times 10^6/\mu L$)	3.65 ± 0.53	(2.46-4.93)
Hemoglobin (g/dL)	11.6 ± 1.8	(7.2-16.0)
Platelet ($\times 10^6/\mu L$)	148 ± 108	(42-550)
AFP (ng/mL)	229749.3 ± 58110.3	(3.2-6027700.0)
PIVKA-II (AU/mL)	18336.1 ± 58110.3	(12.0-259000.0)

AFP: α-fetoprotein

PIVKA-II: protein induced by Vitamin K absence or antagonist-II

Table2. *Characteristics of the patients in this study.*

Patiant No.	Cause of HCC	Stage of HCC	Child–Pugh Classification	Doses (mg/day)	Concentration(mg/L)
1	Hepatitis C	III	A	400	3.90 ± 0.12
2	Alcoholic hepatitis	IVa	A	800	6.11 ± 0.65
3	Hepatitis B	IVb	A	400	6.89 ± 0.47
4	Alcoholic hepatitis	IVb	A	400	2.36 ± 0.15
5	Alcoholic hepatitis	IVb	A	400	8.04 ± 0.43
6	Hepatitis C	IVb	A	400	17.52 ± 1.13
7	Hepatitis C	II	A	400	13.48 ± 0.90
8	Hepatitis C	IVb	A	400	6.12 ± 0.63
9	Alcoholic hepatitis	III	B	200	16.38 ± 1.19
10	Hepatitis C	IVa	A	200	1.67 ± 0.18
11	Hepatitis C	III	B	200	0.73 ± 0.04
12	Hepatitis B	IVa	B	200	1.38 ± 0.12
13	Hepatitis C	IVa	A	400	5.29 ± 0.29
14	Alcoholic hepatitis	IVa	A	800	4.73 ± 0.82
15	Hepatitis C	IVa	A	400	7.83 ± 1.08
16	Hepatitis C	IVa	A	200	6.66 ± 0.54
17	Hepatitis C	IVb	A	400	2.91 ± 0.32
18	Hepatitis C	IVa	A	200	1.44 ± 0.18
19	Hepatitis C	IVa	A	200	3.61 ± 0.47
20	Hepatitis C	IVa	A	200	3.86 ± 0.41
21	Hepatitis C	IVb	A	400	5.87 ± 0.55
22	Hepatitis C	III	B	200	6.36 ± 0.62
23	Hepatitis C	IVa	B	400	3.71 ± 0.42
24	NASH	IVb	A	200	3.22 ± 0.33
25	Hepatitis C	III	B	200	1.13 ± 0.13
26	Alcoholic hepatitis	IVa	B	800	2.22 ± 0.27
27	Hepatitis B	IVb	A	400	8.86 ± 0.71
28	Hepatitis C	IVb	A	400	3.24 ± 0.56
29	Hepatitis B	IVb	A	400	1.43 ± 0.34
30	Hepatitis C	III	A	400	9.37 ± 1.68
31	Hepatitis C	IVb	A	400	4.17 ± 0.68
32	Hepatitis B	IVb	B	400	1.02 ± 0.19
33	NASH	IVb	A	400	7.78 ± 0.94
34	Hepatitis C	IVa	A	400	8.52 ± 1.48
35	Hepatitis C	II	A	400	4.47 ± 0.98
36	Hepatitis C	IVa	B	400	2.22 ± 0.12
37	Hepatitis C	II	B	800	3.25 ± 0.56

HCC: hepatocellular carcinoma

Values containing the "±" symbol represent mean ± S.D. values.

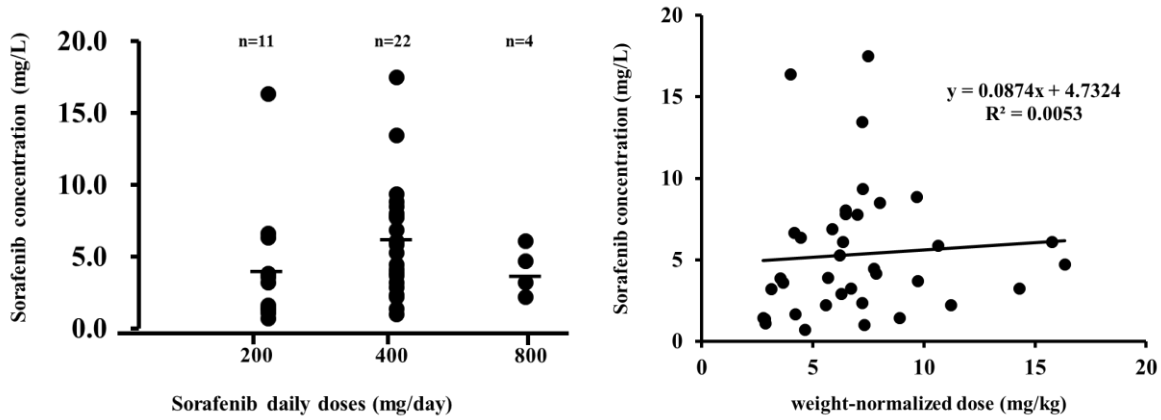

Figure2. *Plasma concentration of sorafenib in patients with HCC. (A) Plasma concentration of sorafenib in patients administered different doses of the drug. (B) Correlation analysis between sorafenib concentrations and the weight-normalized dose; R^2, correlation coefficient.*

Sorafenib is orally administeredfor aHCC. According to the Nexavar interview form, the mean elimination half-life of sorafenib is 25.5 h for a single dose and steady-state plasma sorafenib concentrations could be achieved within 7days. Plasma levels of sorafenib in the steady-state remained constant and $C_{max}$was 4.9 mg/L after 14 days (400 mg/day). Our findings suggested that the serum concentration of sorafenib did not depend upon the oral dose or weight-normalized dose in patients with HCC. The lack of an association between the sorafenib concentration and dose or weight-normalized dose may be attributable to several reasons, such as individual differences and various modes of pathogenesis. In the sorafenib absorption process, the low plasma concentration of sorafenib is attributable tothe presence of portal hypertension, such as hepatic cirrhosis,which reduces absorption efficiency. Sorafenib is metabolized via oxidative metabolism mediated by the 3A4 isoform of cytochrome P450 (CYP3A4), and by glucuroninic acid conjugation mediated by uridine diphosphate glucuronyl transferase 1A9 (UGT1A9) activity in the liver [12]. Many studies have established that single nucleotide polymorphisms (SNPs) of CYP3A4 and UGT1A9 can affect drug metabolism in the liver [13]. Furthermore, Wei and colleagues noted that SNPs in multidrug resistance-associated protein 2 (MRP2) can affect the efflux of sorafenib [14]. Tandiaand coworkers reported that polymorphisms in P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2) were significantly associated with low sorafenib plasma levels [15].

In contrast, sorafenib undergoes enterohepatic recycling [16]. Accordingly, disorders of the defluxion of sorafenib from the liver to the intestine, such as biliary tract obstruction, or absorption from the intestine, such as portal vein thrombosis, may lead to the failure to control sorafenib concentrations in patients with HCC. Hypoalbuminemia or the presence of ascitic fluid may affect the pharmacokinetics of sorafenib as the protein binding capacity of sorafenib in serum shows very high affinity (Nexavar interview form).

Further studies of the regulation of sorafenib concentrations would advance the field of HCC chemotherapy.

In this present study, we found that the concentration of sorafenib showed no significant correlation with the drug dose or weight-normalized dose. Methods aimed at determining sorafenib concentrations are important for HCC chemotherapy because it is necessary to maintain an effective concentration of sorafenib. Hopefully, the outcome of this present study will inform future developments in HCC chemotherapy.

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