BMC Cancer

The change rate in serum nitric oxide may affect lenvatinib therapy in hepatocellular carcinoma

Atsushi Kawamura[1], Haruki Uojima[2,3]*, Makoto Chuma[4,5], Xue Shao[2], Hisashi Hidaka[2], Takahide Nakazawa[2,6], Akira Take[7], Yoshihiko Sakaguchi[7], Kazushi Numata[5], Makoto Kako[3], Akito Nozaki[5], Shintaro Azuma[1], Kazue Horio[2], Chika Kusano[2] and Koichiro Atsuda[1,8]

Abstract

Background: Lenvatinib is appropriate for reducing the production of nitric oxide (NO) and facilitating as block angiogenesis. However, to our knowledge, there are no data that support the correlation between NO and clinical response in patients who received lenvatinib therapy for HCC. Therefore, we investigated the correlation between the change rate of NO levels and clinical responses including adverse events (AEs) after lenvatinib therapy for unresectable hepatocellular carcinoma (HCC).

Methods: This study was conducted using previously collected data from another study. We enrolled 70 patients who received lenvatinib for advanced or unresectable HCC. NO was measured by converting nitrate (NO_3^-) to nitrite (NO_2^-) with nitrate reductase, followed by quantitation of NO_2^- based on Griess reagent. To determine whether lenvatinib influences NO in unresectable HCC, we evaluated the influence of the change rate of NO from baseline after administration of lenvatinib on maximal therapeutic response and SAE.

Results: After lenvatinib administration, a change rate in the NO from 0.27 to 4.16 was observed. There was no difference between the clinical response to lenvatinib and the change rate of NO ($p = 0.632$). However, the change rate of NO was significantly lower in patients with AEs than in those without AEs ($p = 0.030$). When a reduction in NO rate of < 0.8 was defined as a clinically significant reduction of NO (CSRN), the CSRN group had significantly worse progression-free survival (PFS) and overall survival (OS) than the non-CSRN group ($p = 0.029$ and $p = 0.005$, respectively).

Conclusion: Decreased NO levels were associated with the occurrence of AEs and worse prognosis after lenvatinib administration. Change rate in serum NO can be used as predictive markers in patients receiving lenvatinib therapy for HCC.

Keywords: Nitric oxide, Lenvatinib therapy, Hepatocellular carcinoma, Adverse effects, Progression-free survival, Overall survival

*Correspondence: kiruha@kitasato-u.ac.jp

[2] Department of Gastroenterology, Internal Medicine, Kitasato University School of Medicine, 1-15-1 Kitasato, Minami-ku, Sagamihara, Kanagawa 252-0375, Japan
Full list of author information is available at the end of the article

Introduction

Malignant tumors require the formation of mature blood vessels to promote their growth and contribute to pathological processes in the tumor environment [1, 2]. Nitric oxide (NO), a simple gas with divergent biological activities, seems to play a crucial role in angiogenesis [3]. The tumor-promoting effect of NO is understood as a

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convergence of diverse signaling mechanisms with prominent pathways such as NO synthase (NOS)-derived NO and vascular endothelial growth factor (VEGF) [4, 5].

VEGF is secreted by tumor cells in response to hypoxia. Exposure of endothelial cells to VEGF leads to the phosphorylation and activation of NOS, resulting in the conversion of L-arginine and molecular oxygen into L-citrulline and NO [6]. Thus, VEGF elevation has been extensively reported to correlate with angiogenesis and tumor progression [7]. VEGF expression correlates with the degree of tumor vascularization and increased metastatic risk [5].

On this basis, simultaneously suppressing VEGF signals suppress tumor angiogenesis to the cancer cells, and VEGF inhibitors appropriately reduce the production of NO and facilitate antitumor drug delivery as block angiogenesis [8, 9]. Lenvatinib, a novel multikinase inhibitor that targets VEGF receptors, reduces NO production by reducing the activity of angiogenic factor-mediated pathways [10, 11]. A randomized phase III non-inferiority trial showed lenvatinib was non-inferior to sorafenib in overall survival (OS) for the patients with unresectable hepatocellular carcinoma (HCC) [12].

Assuming more dramatic effects in unresectable HCC, the response to this drug is unpredictable. However, a previous study suggested that elevated NO levels in HCC patients were significantly reduced after radiofrequency ablation [13]. Lenvatinib reduce NO production by reducing the activity of NOS-derived NO and VEGF. Therefore, we hypothesized that there was a positive correlation between the reduction of the NO levels and the therapeutic effect of lenvatinib for HCC. Furthermore,

NO is a vital molecule that contributes to numerous physiological phenomena in various biological systems [14]. Therefore, severe reduction of NO levels can lead to adverse events (AEs) from simultaneous suppression of vital organs after lenvatinib therapy. However, to our knowledge, there are no data that support the correlation between NO and a clinical response in patients who received lenvatinib therapy for HCC. Therefore, we investigated the correlation between the change rate of the NO levels and the clinical responses including adverse events (AEs) after lenvatinib therapy for unresectable hepatocellular carcinoma (HCC).

Methods

Ethics

This study was approved by the Institutional Review Boards and Ethics Committees of all hospitals involved (IRB number: 11000845). The study was registered in the Japan Registry of Clinical Trials (jRCT ID: 1030210283). These data were previously collected under another study (UMIN ID: 000036625). The data were collected after each patient wrote informed consent for the treatment.

Patients

This study was performed using previously collected data under another study that was conducted across three medical institutions in Japan from 2017 to 2020 [15]. The preliminary study enrolled 168 patients aged > 20 years who received lenvatinib for advanced or unresectable HCC (Fig. 1). Of those, 68 patients were excluded due to the following exclusion criteria: (i) lenvatinib discontinued within 14 days, (ii) malignancies other than HCC,
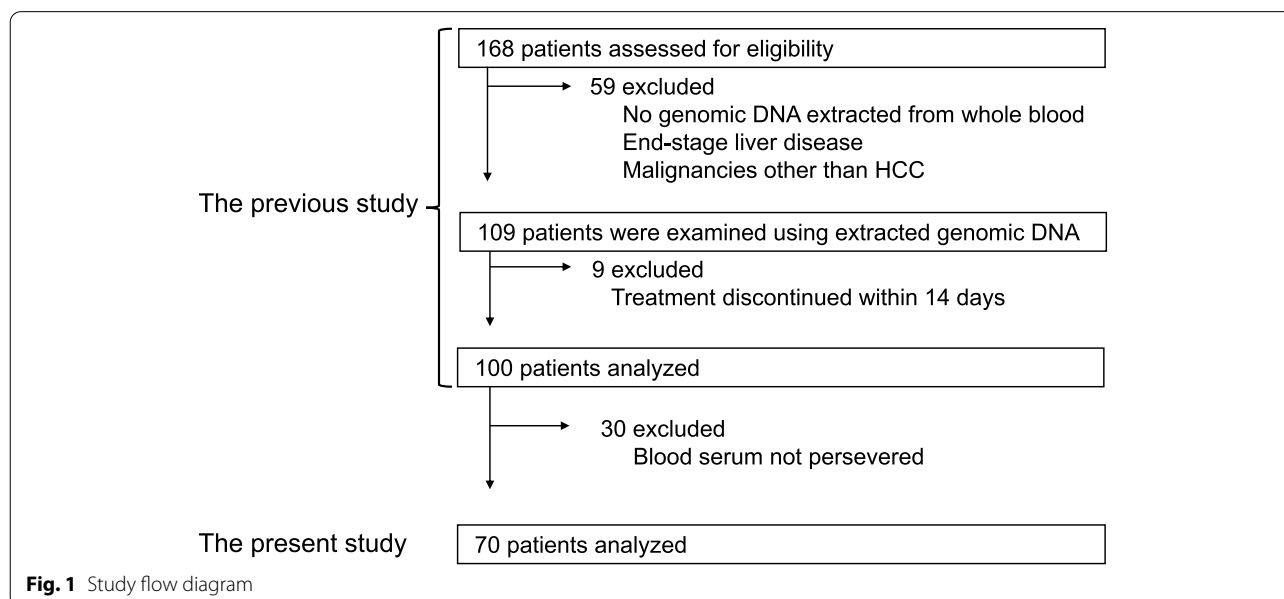

Fig. 1 Study flow diagram

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(iii) no genomic DNA extracted from blood, and (iv) end-stage liver failure. Therefore, the previous study analyzed 100 patients [15]. Of those patients, 30 did not receive blood serum to identify the biomarkers of the response of HCC to lenvatinib. Therefore, relevant clinical data were collected from the remaining 70 patients.

Diagnosis of HCC and recommendation of lenvatinib

HCC diagnosis was based on imaging modalities such as computed tomography and magnetic resonance imagery. Liver tumors with atypical imaging findings were histopathologically analyzed by biopsy. Lenvatinib was administered for unresectable or advanced HCC that was characterized by vascular invasion, metastatic disease, and/or progression after locoregional treatments.

A starting drug dose was dependent on the patient's weight: 12 mg and 8 mg/day for body weight $\geq$ 60 kg and < 60 kg, respectively. However, for patients with risk factors, such as low PS (performance status), Child-Pugh class B, and sarcopenia, an approved dose reduction from the initial dose depended on the attending physician's discretion.

NO measurement

Blood samples, collected at a pretreatment visit and within a month after administration of Lenvatinib [16], were centrifuged at 25°C (room temperature) at 3000 rpm for 5 min. The fractionated serum was stored at -45°C. NO was measured by converting nitrate (NO_3^-) to nitrite (NO_2^-) with nitrate reductase, followed by quantitation of NO_2^-, using a Griess reagent. In this experiment, we used a colorimetric Nitric Oxide Assay kit (Oxford Biomedical Research, MI, USA). Absorbance was measured at 540 nm using a microplate reader (see Additional file 1). We measured the change levels and rate in NO from baseline to after administration of lenvatinib. The change rate in NO was calculated as in the NO levels after lenvatinib therapy and compared with the previous NO levels.

End-point measurement

The end-points were the influence to the maximal therapeutic response and Serious AE (SAE) from the change levels and rate in NO after administration of lenvatinib. The maximal therapeutic response was evaluated by the modified Response Evaluation Criteria in Solid Tumors (mRECIST). SAEs were defined as events that result in death, are life threatening, require inpatient hospitalization or prolongation of existing hospitalization, or result in persistent or significant disability/incapacity according to ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use).

Statistical analyses

All *p*-values were two-sided, with differences < 0.05 considered to indicate statistical significance. Two categorical variables in the population were examined using the Chi-square test. The differences in OS and progression-free survival (PFS) were evaluated with the log-rank test and the Kaplan-Meier method in the two groups. PFS and OS were analyzed by the Cox proportional hazards regression model. The correlation between NO levels and the response to lenvatinib was analyzed by calculating the odds ratio and 95% confidence interval (CI) using univariate and multivariate logistic regression analyses. All analyses were performed using SPSS (version 24.0; IBM Corporation, Armonk, NY, USA). Statistical analyses were reviewed by the Statista Corporation, Kyoto, Japan.

Results

Patients' characteristics

Table 1 shows the patients' baseline characteristics. The mean age was 71.5 ± 8.9 years, and 53 of 70 patients (75.7%) were male. The mean body weight was 61.5 ± 12.2 kg. The causes of chronic liver disease were virus (*n* = 38), nonalcoholic fatty liver disease (*n* = 16), alcohol (*n* = 10), and others (*n* = 6). There were 46 patients (65.7%) with liver cirrhosis. Lenvatinib was administered for vascular invasion (*n* = 24), metastatic disease (*n* = 22), and progression after locoregional treatment (*n* = 46). The types of treatment given were radiofrequency ablation (RFA) (*n* = 28), transcatheter arterial therapy (*n* = 50), and molecular targeted therapy (*n* = 6). There were 32 (45.7%) and 38 (54.3%) patients who were diagnosed as having Barcelona Clinic Liver Cancer (BCLC) stages B and C, respectively. There were 25, 35, and 10 patients who had daily initial doses of 12 mg, 8 mg, and 4 mg, respectively. The 10 patients, who received an initial dose of 4 mg, had liver cirrhosis with sarcopenia.

Clinical responses of patients who received lenvatinib

The numbers of complete response, partial response, stable disease, progressive disease (PD), and unevaluated response in maximal therapeutic response were 2 (2.9%), 24 (34.2%), 25 (35.7%), 15 (28.0%), and 4 (5.7%), respectively. The median PFS and OS in all patients were 163 and 403 days (95% CI 124–238 and 311–524 days), respectively. The median time to treatment failure values for all patients was 247 days (95% CI: 201–292). Reasons to discontinue treatment were PD (*n* = 40), unmanageable AEs (*n* = 26), and withdrawal at the patients' own discretion (*n* = 4). Table 2 shows the treatment-related severe AEs in the study period. After discontinuation of lenvatinib therapy, administration of another molecularly

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Table 1 Baseline clinical characteristics

		N	70
Age	yrs		71.5 ± 8.9
Gender: Male	n (%)		53 (75.7)
Etiology: HBV/HCV/Alcohol/NASH/etc.	n		10/28/10/16/6
Performance status: 0/1	n		65/5
Child-Pugh score 5:6:7:8:9	n		39:17:9:4:1
Child-Pugh class: A/B	n		56/14
Weight	kg		61.5 ± 12.2
< 60 kg/≥ 60 kg			37/33
Body mass index	kg/m^2		23.3 ± 4.6
Barcelona Clinic Liver Cancer stage: B/C	n		32/38
Macroscopic portal vein invasion: Yes/No	n		24/46
Extrahepatic spread: Yes/No	n		22/48
Up to 7: In/Out	n		36/34
Tumor size:	mm		47.1 ± 38.9
Previous therapy:			
Radiofrequency ablation	n		28
Transcatheter treatment	n		50
Molecularly-targeted therapy	n		6
Initial dose of lenvatinib: 4 mg/8 mg/12 mg	n		10/35/25
Hemoglobin	g/dL		12.2 ± 2.12
Platelets	$\times 10^4$/μl		14.1 ± 6.3
Prothrombin time	%		87.1 ± 19.3
Serum albumin	g/dL		3.7 ± 0.45
BUN	g/dL		20.1 ± 14.3
Serum creatinine	mg/dL		0.86 ± 0.36
Aspartate aminotransferase	IU/L		52.0 ± 30.3
Alanine aminotransferase	IU/L		34.0 ± 23.8
Total bilirubin	g/dL		0.9 ± 0.41
Ammonia	μg/dl		51.2 ± 43.0
α-fetoprotein	ng/mL		$12,309 \pm 49,567$
PIVKA-II	mAU/mL		$6083 \pm 14,136$

Table 2 Severe adverse events

Severe AE	CSRN: $n = 25$	Non-CSRN: $n = 45$
Decreased appetite	6 (24.0)	4 (8.8)
Hepatic ascites	4 (16.0)	3 (6.6)
Hepatic encephalopathy	3 (12.0)	2 (4.4)
Gastrointestinal bleeding	2 (8.0)	–
Proteinuria	2 (8.0)	1 (2.2)
Increased blood bilirubin	1 (4.0)	1 (2.2)
Acute pancreatitis	1 (4.0)	1 (2.2)
Rhabdomyolysis	1 (4.0)	–
Sepsis	1 (4.0)	–
Gastrointestinal perforation	–	1 (2.2)
Interstitial pneumonia	–	1 (2.2)

targeted therapy, transcatheter treatment, and best supportive care were performed for 18 (25.7%), 8 (11.4%), and 29 (41.4%) patients, respectively.

Pretreatment NO levels

The mean NO level at baseline was 49.2 ± 39.8 nmol/mL. We analyzed the correlation between the NO levels and baseline characteristics (see Additional file 2). BCLC stages B and C were 48.3 ± 40.5 and 50.0 ± 39.8, respectively. Up to 7 in and out were 46.6 ± 39.8 and 52.0 ± 40.6, respectively. The NO levels in patients with and without high blood pressure (HBP) were 49.3 ± 41.2 and 49.1 ± 39.3, respectively. No significant differences were found among age, sex, body weight, etiology, tumor size, HBP, BCLC stage, and presence of portal invasion.

Change levels and rate of NO after lenvatinib therapy

Lenvatinib reduced the NO levels in 39 (55.7%) patients. After lenvatinib administration, change levels in the NO from 49.2 ± 39.8 to 45.1 ± 32.5 nmol/mL were observed ($p = 0.193$) (Fig. 2A) and a change rate in the NO was observed from 0.27 to 4.16 (Fig. 2B).

Tumor response in the change levels and rate of NO after administration of lenvatinib

Non-PD and PD were -4.4 ± 28.5 and -3.2 ± 19.9 in the change levels of the NO. There was no difference between clinical response to lenvatinib therapy and change levels of the NO ($p = 0.864$) (Fig. 3A). Non-PD and PD were 1.3 ± 0.8 and 1.2 ± 0.7, in the change rate of the NO. There was no significant difference between clinical response to lenvatinib therapy in HCC and change rate of the NO ($p = 0.632$) (Fig. 3B).

SAE in the change levels and rate of NO after administration of lenvatinib

The change levels of the NO in the patients with the absence and presence of SAE were 1.12 ± 30.6 and -9.67 ± 19.6. The change levels of NO was lower in patients with AEs than in those without SAE ($p = 0.085$) (Fig. 3C). The change rate of the NO in the patients with the absence and presence of SAE were 1.4 ± 0.9 and 1.0 ± 0.52, respectively. The change rate of NO was significantly lower in patients with SAE than in those without SAE ($p = 0.030$) (Fig. 3D).

Correlation between HBP and change of NO after lenvatinib therapy

The change levels of NO in patients with and without HBP were -4.1 ± 18.1 and -3.9 ± 21.9, respectively. No
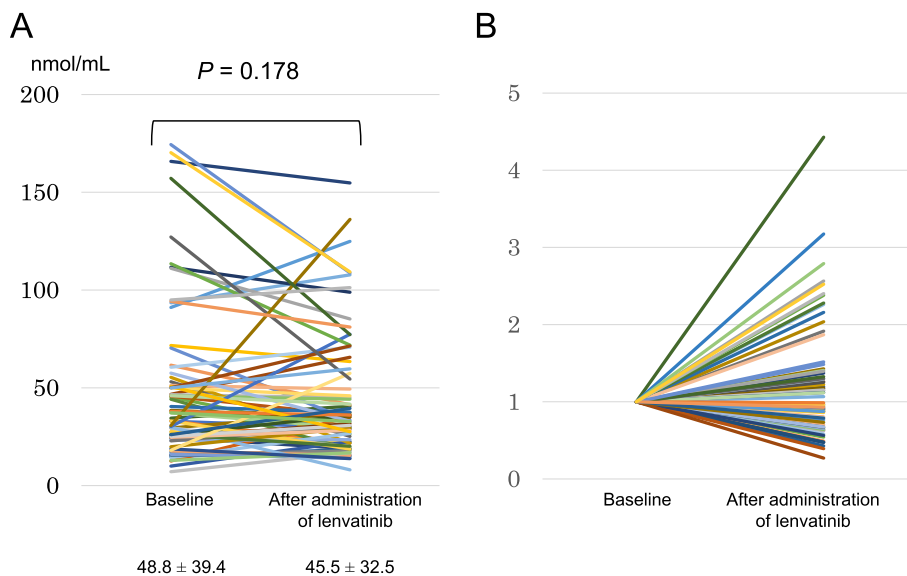
Fig. 2 A Change levels of NO after lenvatinib therapy "pre" indicates before lenvatinib administration; "post" indicates after lenvatinib administration. **B** Change rate of NO after lenvatinib therapy "pre" indicates before lenvatinib administration; "post" indicates after lenvatinib administration

significant correlation was found between the presence of HBP and change levels of NO ($p = 0.561$). Furthermore, the change rate of NO in patients with and without HBP were 1.1 ± 1.0 and 1.2 ± 0.9, respectively. No significant correlation was found between the presence of HBP and change rate of NO ($p = 0.813$).

PFS and OS based on reduced NOS levels

Receiver-operating characteristic (ROC) curve analysis was performed to assess the occurrence of SAE in patients with HCC. The respective cut-off points for SAE after lenvatinib treatment were estimated using ROC curves for the change rate of the NO (see Additional file 3). Using a cut-off for the reduction of 0.8, predicting the occurrence of AEs had a sensitivity of 77.3% and a specificity of 42.3%. A reduction in NO rate of < 0.8 was defined as a clinically significant reduction of NO (CSRN).

The median PFS in the CSRN and non-CSRN groups was 131 days and 238 days (95% CI 91–166 days and 130–377 days), respectively. Patients in the CSRN group experienced significantly worse PFS than those in the non-CSRN group (log-rank test for trend: PFS, $p = 0.029$) (Fig. 4A).

The median OS in the CSRN and non-CSRN groups was 311 and 524 days (95% CI 185–421 days and 336–853 days), respectively. Patients in the CSRN group experienced significantly worse OS than those with
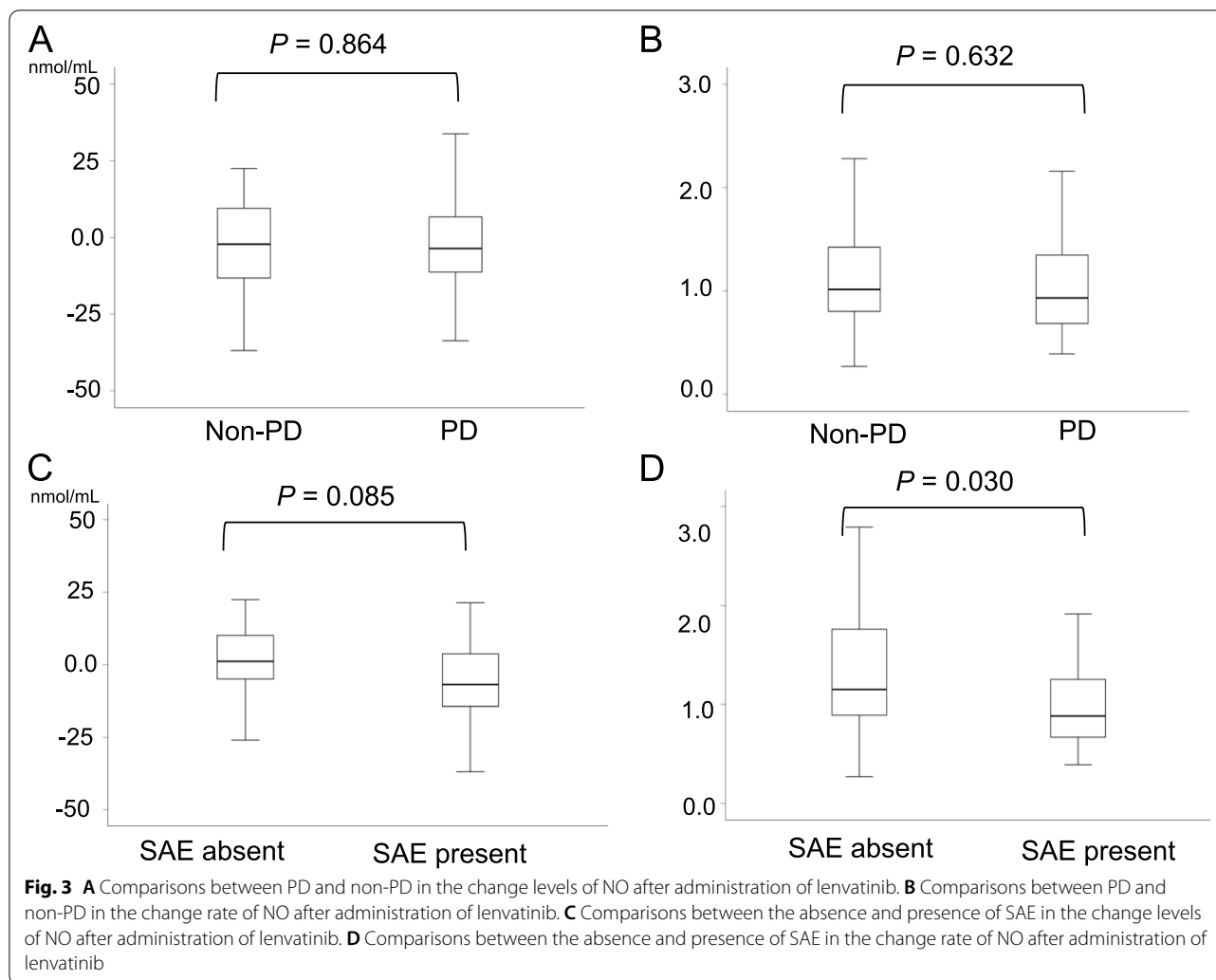
non-CSRN (log-rank test for trend: OS, $p = 0.005$) (Fig. 4B).

Univariate and multivariate analysis of factors affecting PFS and OS

Table 3 shows the risk factors associated with PFS using a cox proportional-hazards regression mode. In multivariate analysis, presence of CSRN were significantly associated with PFS (hazard ratio [HR] 1.848; 95% CI 1.05–3.24; $p = 0.032$). Table 4 shows the risk factors associated with OS using a cox proportional-hazards regression mode. In multivariate analysis, albumin and presence of CSRN were significantly associated with OS (HR 2.401; 95% CI 1.073–5.370; $p = 0.033$ and HR 2.107; 95% CI 1.130–3.599; $p = 0.018$, respectively).

Discussion

To our knowledge, this is the first study to report the influence of NO levels after administration of lenvatinib regarding the clinical response and AEs for patients with unresectable HCC. A review clearly showed a significant reduction in NO levels after RFA for HCC [14]. Therefore, we expected a correlation between the change in NO levels and the patients' responses to lenvatinib. In fact, a few cases showed positive correlations between increased NO levels and disease progression after the lenvatinib administration. Disease progression may be seen if HCC has higher production of serum NO levels than the restored NO production due to the lenvatinib

Fig. 3 **A** Comparisons between PD and non-PD in the change levels of NO after administration of lenvatinib. **B** Comparisons between PD and non-PD in the change rate of NO after administration of lenvatinib. **C** Comparisons between the absence and presence of SAE in the change levels of NO after administration of lenvatinib. **D** Comparisons between the absence and presence of SAE in the change rate of NO after administration of lenvatinib

therapy. However, there were no statistically significant correlations observed between the patients' responses and the NO levels. The major difficulties in investigating the physiological role of NO is the direct measurement of NO due to its short lifetime and very low concentrations [17]. Therefore, most researchers refer to indirect qualitative measurements, such as the detection of NO-induced physiological AEs and employment of NOS inhibitors [18]. In this experiment, the Griess method based on the chemical diazotization reaction to detect NO, which is the indirect method based on two stable breakdown products, NO_3^- and NO_2^-, can be easily detected by photometric means [19]. However, quantification by absorbance is affected by thiols and proteins. As a result, the indirect measurement may be slightly less rigorous than the direct measurement. In addition, we measured systemic NO in whole blood, including NO from tumor cells, hepatic sinusoidal endothelial cells, and vascular epithelial cells. The results of the present study

may be different from those using real-time NO levels in tumor cells [20, 21].

On the other hand, the present study revealed that a significant reduction in the NO rate was associated with AEs after the lenvatinib therapy. Lenvatinib therapy improves HCC control by restoring the NO levels in tumor. However, excessively restored NO levels lead to the occurrence of AEs and shortened lengths of survival. Inhibition of angiogenic factor-mediated pathways, including VEGF, results in a subsequent reduction in NO production [22]. NO is an important vasodilator that maintains vascular tone by activating guanylate cyclase in vascular smooth muscle [23]. In hepatic microcirculation, the deficiency of endothelial NO release causes hemodynamic abnormalities and portal hypotension according to the progression of fibrosis in chronic liver disease [24]. As a result, a significant reduction in NO appears to promote the occurrence of AEs in anti-angiogenic therapy. In fact, our team revealed that lenvatinib
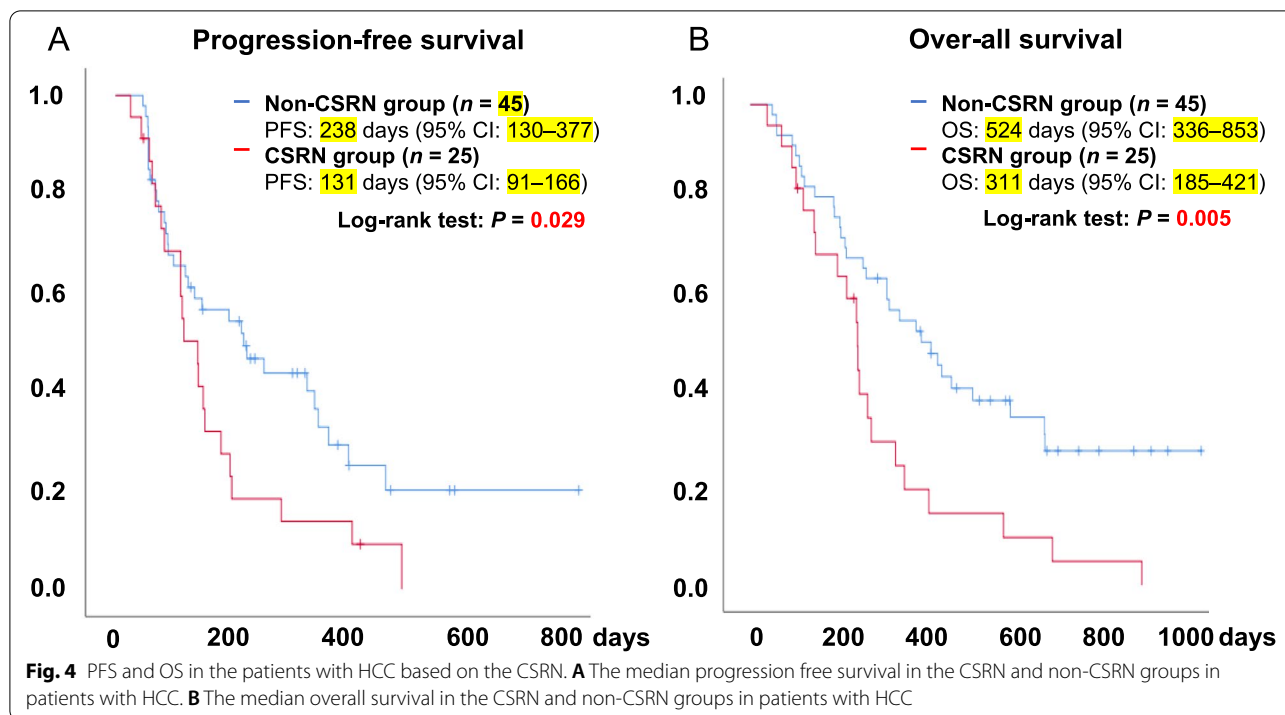
Fig. 4 PFS and OS in the patients with HCC based on the CSRN. **A** The median progression free survival in the CSRN and non-CSRN groups in patients with HCC. **B** The median overall survival in the CSRN and non-CSRN groups in patients with HCC

Table 3 Univariate and multivariate analyses of factors affecting PFS

Variable		Univariate analysis		Multivariate analysis	
		HR (95% CI)	*P* value	HR (95% CI)	*P* value
CSRN	–	1.000		1.000	
	+	1.848 (1.054–3.242)	0.032	1.848 (1.054–3.242)	0.032
Age	< 70	1.000			
	≥ 70	1.812 (0.991–3.311)	0.054		
Body weight (kg)	< 60	1.000			
	≥ 60	1.089 (0.627–1.894)	0.761		
Barcelona Clinic Liver Cancer stage	B	1.000			
	C	1.498 (0.864–2.597)	0.150		
Up to seven	In	1.000			
	Out	1.243 (0.719–2.148)	0.436		
Metastatic disease	–	1.000			
	+	1.247 (0.703–2.214)	0.450		
Previous therapy: Transcatheter treatment	–	1.000			
	+	1.412 (0.761–2.623)	0.274		
Refractory to Transcatheter treatment	–	1.000			
	+	1.389 (0.501–2.123)	0.360		
α-fetoprotein (ng/mL)	< 400	1.000			
	≥ 400	1.100 (0.620–1.950)	0.745		
Albumin	≥ 3.2	1.000			
	< 3.2	2.130 (0.992–4.574)	0.052		

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Table 4 Univariate and multivariate analyses of factors affecting overall survival

Variable		Univariate analysis		Multivariate analysis	
		HR (95% CI)	*P* value	HR (95% CI)	*P* value
CSRN	–	1.000		1.000	
	+	2.203 (1.253–3.873)	0.006	2.017 (1.130–3.599)	0.018
Age	< 70	1.000			
	≥ 70	1.690 (0.926–3.085)	0.088		
Body weight (kg)	< 60	1.000			
	≥ 60	1.130 (0.652–1.960)	0.663		
Barcelona Clinic Liver Cancer stage	B	1.000			
	C	1.071 (0.616–1.861)	0.808		
Up to seven	In	1.000			
	Out	1.450 (0.838–2.510)	0.184		
Metastatic disease	–	1.000			
	+	1.042 (0.576–1.886)	0.891		
Previous therapy: Transcatheter treatment	–	1.000			
	+	1.203 (0.650–2.229)	0.556		
Refractory to Transcatheter treatment	–				
	+	1.311 (0.596–1.520)	0.742		
α-fetoprotein (ng/mL)	< 400	1.000			
	≥ 400	1.630 (0.920–2.885)	0.094		
Albumin	≥ 3.2	1.000		1.000	
	< 3.2	2.866 (1.309–6.276)	0.008	2.401 (1.073–5.370)	0.033

aggravates portal hypertension using duplex Doppler ultrasonography [25].

We also expected a correlation between the change in NO levels and HBP. Previous studies have shown that patients who experienced hypertension after lenvatinib administration had significantly better outcomes than those who did not develop AEs [15]. A subsequent reduction in NO production lead to HBP. However, no correlation was observed between the occurrence of HBP and NO levels in the present study. Some patients in the present study had HBP. To provide treatment for the HBP may have masked the HBP caused by the lenvatinib therapy.

Thus, the present study revealed the correlation between the change rate of NO and SAEs in patients with HCC who underwent the lenvatinib therapy. However, as evidenced previously, the distinct roles of NO in patients treated with VEGF inhibitors remain unclear [26]. Previously, the relationship between NO levels and tumor progression has been linked to the presence of NOS enzymes in cells and/or serum. Therefore, NOS should be considered for patients with HCC who have received lenvatinib therapy. NOS is classified into three subgroups: neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). VEGF inhibitors mainly block the exposure of endothelial cells to VEGF, leading to the activation of eNOS, which is a signaling mechanism with prominent pathways in angiogenesis [27, 28]. eNOS is mainly expressed in liver sinusoidal endothelial cells and vascular epithelial cells, including the hepatic artery, central veins, and portal vein. The most common cause of portal hypertension is an increase in intrahepatic vascular resistance through the production of eNOS-derived NO [29]. The excessive reduction of eNOS by lenvatinib leads to AEs. Furthermore, it will be necessary to verify the influence of iNOS on the HCC response in future studies. Tumor cells are the primary sites for excess NO production, and the amount of NO produced by iNOS, which is higher than that produced by nNOS or eNOS, contributes to tumor cell-related angiogenesis, malignant transformation, invasion, and metastasis [30, 31]. The overproduction of NO in malignant tissues by iNOS inhibits the immune defense mechanism and increases tumor blood, correlating with carcinogenesis and playing a role in tumor progression in HCC [32, 33]. Therefore, it is necessary to consider not only NO but also NOS in patients with HCC.

There are a few limitations to this study. First, it was a retrospective study. Second, preserved blood serum samples could not be obtained on a scheduled day from all the patients receiving lenvatinib. Furthermore, we need to evaluate the most appropriate time after

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administration of lenvatinib in which AEs occur. Third, NO was indirectly assessed using nitrate NO_3^- and nitrite NO_2^-. Evaluation of NO by direct methods will be needed for future analysis. We only evaluated the correlation between the change rate of the NO levels and the clinical responses and AEs in the present study. Furthermore, this study did not exclude the effect on NO concentrations from any treatment given before the lenvatinib administration. There is insufficient evidence in the literature on the mechanism of how NO affects the therapeutic response and AEs. Fourth, patients with a total bilirubin of 2.0 mg/dL or higher were excluded because of the effect on absorbance measurements.

Conclusion

Decreased NO levels were associated with the occurrence of AEs and worse prognosis after the lenvatinib administration. Change rate in serum NO levels can be used as predictive markers in patients receiving lenvatinib therapy for HCC.

Abbreviations

NO: Nitric oxide; AE: Adverse event; HCC: Hepatocellular carcinoma; NO_3^-: Nitrate $_3^-$; NO_2^-: Nitrite $_2^-$; CSRN: Clinically significant reduction of nitric oxide; PFS: Progression-free survival; OS: Overall survival; NOS: Nitric oxide synthase; nNOS: Neuronal nitric oxide synthase; iNOS: Inducible nitric oxide synthase; eNOS: endothelial nitric oxide synthase; VEGF: Vascular endothelial growth factor; mRECIST: modified Response Evaluation Criteria in Solid Tumor; CI: Confidence interval; RFA: Radiofrequency ablation; BCLC: Barcelona clinic liver cancer; PD: Progressive disease; HBP: High blood pressure; ROC: Receiver-operating characteristic; ALBI: Albumin-bilirubin.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-022-10002-x.

Additional file 1. The measurement of NO Production of NO by NOS and its metabolism to nitrate and nitrite. Nitrate was converted to nitrite by nitrate reductase, and NO was measured using the Griess reagent. The microtiter plate after the reaction is shown. (PPTX 2781 kb)

Additional file 2. Pretreatment NO levels

Additional file 3. ROC. The occurrence of SAE using lenvatinib by ROC curves for patients with HCC. AUC, area under the curve. (PPTX 48 kb)

Authors' contributions

Atsushi Kawamura is responsible for the concept and design, collection and assembly of data, and statistical analysis. Haruki Uojima and Makoto Chuma collected and assembled the data. Hisashi Hidaka and Takahide Nakazawa analyzed and interpreted the data. Makoto Kako was the general director of the study group. Atsushi Kawamura, Haruki Uojima, Makoto Chuma, Xue Shao, Hisashi Hidaka, Takahide Nakazawa, Akira Take, Yoshihiko Sakaguchi, Kazushi Numata, Makoto Kako, Akito Nozaki, Shintaro Azuma, Kazue Horio, Chika Kusano, Koichiro Atsuda wrote and gave final approval to the manuscript.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Boards and Ethics Committees of all hospitals involved. The Kitasato University School of Medicine and Hospital Ethics Committee (CRB3180002, B20–253), Yokohama City University Clinical Research Review Committee (CRB318007, A210100017). The study was registered in the Japan Registry of Clinical Trials (jRCT ID: 1030210283). These data were previously collected under another study (UMIN ID: 000036625). The data were collected after each patient wrote informed consent for the treatment and written informed consent was obtained from all participants. All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests as defined by Nature Research, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

Author details

[1]Department of Pharmacy, Kitasato University Hospital, Sagamihara, Kanagawa, Japan. [2]Department of Gastroenterology, Internal Medicine, Kitasato University School of Medicine, 1-15-1 Kitasato, Minami-ku, Sagamihara, Kanagawa 252-0375, Japan. [3]Gastroenterology Medicine Center, Shonan Kamakura General Hospital, Kamakura, Kanagawa, Japan. [4]Department of Gastroenterology, Yokohama City University Hospital, Yokohama, Kanagawa, Japan. [5]Gastroenterological Center, Yokohama City University Medical Center, Yokohama, Kanagawa, Japan. [6]Nakazawa Internal Medicine Clinic, Sagamihara, Kanagawa, Japan. [7]Department of Microbiology, Kitasato University School of Medicine, Sagamihara, Kanagawa, Japan. [8]School of Pharmaceutical Sciences, Kitasato University, Shirokane, Minato-ku, Tokyo, Japan.

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