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Transarterial chemoembolization with sorafenib in VX2 tumor model of rabbit liver: pharmacokinetics and antitumor effect

토끼 VX2 간종양 모델에서 소라페닙을 이용한 경동맥화학색전술: 약동학 및 항종양 효과

2016 년 2 월

서울대학교 대학원

의학과 영상의학전공

김 경 민

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지도교수 윤 창 진

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Abstract

Transarterial chemoembolization with sorafenib in VX2 tumor model of rabbit liver: pharmacokinetics and antitumor effect

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Purpose To investigate the feasibility, safety and anti-tumor effect of transarterial chemoembolization (TACE) using sorafenib in VX-2 tumor model of rabbit liver.

Materials and methods Twenty New Zealand white rabbits with VX2 tumor in the liver were divided into two groups, treated with hepatic arterial administration of 0.5mL of iodized oil alone (TAE-L group) and 0.5mL of iodized oil and 10mg of sorafenib (TAE-S group), respectively. Liquid chromatography tandem mass spectrometry was used to measure the concentration of sorafenib in the peripheral blood and the tumor at 0.5, 1, 2, 4, 24, and 72 hours after treatment. Hepatic enzymes, vascular endothelial growth factor (VEGF), and hypoxia-inducible factor 1α (HIF-1α) were measured at 0, 24, and 72 hours after treatment. Histopathologic examination was performed to evaluate the degree of tumor necrosis and normal parenchymal damage.

Results Access of left hepatic artery was possible in all the rabbits and Lipiodol or emulsion of sorafenib and Lipiodol were delivered successfully as planned. Serum sorafenib concentration peaked at 2 hours after treatment and average tissue concentration was 406.8 times higher than serum concentration. Aspartate aminotransferase and alanine aminotransferase level showed transient elevation in TAE-S group at 24 hours after treatment. Mean serum VEGF and HIF-1α concentrations showed no significant difference between two groups. Degree of ballooning degeneration and coagulation necrosis of peritumoral hepatic

parenchyma were more severe in TAE-S group. Mean fraction of tumor necrosis after treatment was significantly higher (p=0.011) in TAE-S group (83.9%) than that in TAE-L group (56.6%).

Conclusion TACE using sorafenib was feasible in VX2 tumor model of rabbits, resulting in high intratumoral concentration of sorafenib. Degree of tumor necrosis was significantly higher in TAE-S group than TAE-L group, but more severe toxicity of normal liver tissue occurred.

Keywords: Sorafenib, chemoembolization, hepatocellular carcinoma, VX2 tumor

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Introduction

Transarterial chemoembolization (TACE) is an established treatment for unresectable hepatocellular carcinoma (HCC), which significantly improves survival. A randomized controlled trial showed that the survival was significantly better in the chemoembolization group (1 year, 57%; 2 year, 31%, 3 year, 26%) than in the symptomatic treatment group (1 year, 32%; 2 year, 11%, 3 year, 3%; p=.002) [9]. It can not only deliver high dose of chemotherapeutic agents to the tumor, but also induce ischemic necrosis by arterial embolization.

Although hypoxia is one of major mechanism of tumor necrosis induced by TACE, this hypoxia also can enhance proliferation, angiogenesis, metastasis, chemoresistance, and radioresistance of HCC, and consequently lead to recurrence of TACE [16]. If TACE does not induce complete necrosis of the tumor, the residual surviving cancerous tissue induces overexpression of vascular endothelial growth factor (VEGF) [14]. Recently, a study comparing prognosis and hypoxia-inducible factor 1α (HIF-1α) between resection-only group and preoperative TACE group has published [18]. The study showed that protein levels of HIF-1α were significantly increased in TACE tissues, and preoperative TACE was significantly associated with increased 2-year recurrence rate (80 vs. 36%, p=0.00402) and shorter disease-free survival time (11.9 vs. 35.7 months, p=0.0182). The authors concluded that preoperative TACE conferred poor prognosis in HCC patients through activation of HIF-1α.

Thus, inhibition of angiogenesis is critical for the treatment of HCC. Sorafenib (Nexavar; Bayer, Leverkusen, Germany) is a multiple receptor tyrosine kinase inhibitor which interrupts signaling pathways involved in tumor progression and angiogenesis [15]. It is the first agent resulting in improved survival of patients with unresectable advanced HCC from 7.9 months to 10.7 months (hazard ratio (HR) 0.69, $p<0.001$) through oral administration [2; 8], and it was approved for treatment of unresectable HCC in late 2007. Despite its survival benefit, sorafenib is responsible for several systemic adverse effects. According to the sorafenib HCC Assessment Randomized Protocol (SHARP) trial, about 40% of the patients suffered from diarrhea and 20% experienced hand-foot skin reaction. Other significantly increased adverse events included weight loss, alopecia, dry skin, anorexia, voice change, and abdominal pain [8]. These adverse events caused dose reductions in 26% and dose interruptions in 44% of sorafenib recipients [8].

Theoretically, combination treatment of sorafenib and TACE can inhibit tumor angiogenesis, reduce tumor recurrence and metastasis. However, up to date, a large number of studies have tried to combine oral administration of sorafenib with TACE and the results were controversial. A systemic review showed that the hazard ratio of time-to-progression (TTP) was found to be 0.76 (95% confidence interval (CI) 0.66-0.89; $p<0.001$), suggesting the combined use of sorafenib and TACE may improve TTP compared with TACE alone in patients with unresectable HCC [5]. However, the HR for overall survival was found to be 0.81 (95% CI 0.65-1.01; $p=0.061$), indicating the combined use of systemic sorafenib plus TACE might not

improve OS compared with TACE alone [5].

Transarterial administration of sorafenib to the liver would prevent the TACE-induced angiogenesis and tumor growth with lowered systemic adverse effects. A few studies on the feasibility of transarterial administration of sorafenib in iodized oil emulsion into the normal liver model have been published [1; 4; 12; 19]. Transarterial sorafenib delivery resulted in higher tissue drug levels than that in systemic sorafenib therapy and high liver-to-serum ratio without immediate biochemical and histopathologic tissue toxicity [1; 4]. Also, there have been few reports regarding pharmacokinetics, safety, and degree of tumor growth following transarterial administration of sorafenib in VX2 tumor model [12; 19]. Zhang et al. [19] focused on pharmacokinetics, and Parvinian et al. [12] dealt with tumor growth rate and intrahepatic metastasis. Thus, the effect on angiogenesis factors such as VEGF and HIF-1α following transarterial administration of sorafenib was not evaluated in the previous studies. Also the degree of tumor necrosis after TACE using sorafenib compared with that after conventional TACE was not studied yet.

So the aim of the current study was to investigate the feasibility and safety of transarterial chemoembolization using sorafenib and the effects on angiogenesis factors and degree of tumor necrosis.

Materials and Methods

Animal Model

This animal study was approved by our institutional animal care and use committee. Twenty male New Zealand white rabbits weighing 2.9-3.4 kg each were used. The VX2 carcinoma strain had been maintained by means of successive transplantation of tumor cells into the hind limbs of the carrier rabbits. Anesthesia was induced through the administration of an intravenous injection of ketamine hydrochloride (50 mg/kg; Ketamine, Yuhan, Korea) and 2% xylazine (0.1 mg/kg; Rompun, Bayer, Germany). After a midline abdominal incision, the left lateral lobe of the liver was exposed. A single $1mm^3$ tumor chip was implanted at a depth of 5mm from capsule of the liver. After implantation, hemostasis was achieved by application of gentle pressure with a cotton swab to allow the growth of a single, well-demarcated tumor in the liver of each recipient rabbit. Chemoembolization was performed two weeks after tumor implantation, when tumors were expected to be a diameter of 2 cm.

Preparation of Sorafenib in Iodized Oil Emulsion

Considering generally adapted maximum dose of iodized oil (10 mL in a single session of TACE) and assuming the weight of a rabbit as 1/20 of human adult, we decided target dose of iodized oil (Lipiodol, Andre Guerbet, Aulnay-sous-Bois,

France) as 0.5 mL. In rabbits, therapeutic plasma sorafenib levels approximating 5 μg/mL may be reasonably attained by using an oral dosing regimen of 30 mg/kg/day [4]. Considering that chemoembolization generally results in local drug concentrations 10-100 times greater than systemic administration [4], intra-arterial sorafenib dose was targeted at 3 mg/kg. Sorafenib tablets (Nexavar, Bayer Pharmaceuticals) were donated by Bayer Pharmaceuticals. The tablets were ground into fine powder and mixed with Lipiodol, to obtain a concentration of 20mg/mL (i.e. 10 mg/0.5 mL/rabbit). The mixture was emulsified by sonication using Q125 sonicator (Qsonica, USA) with 3.2mm probe. Amplitude was 30% and pulse mode with 1 second-on and 1 second-off was applied. Sonication was performed for 15 minutes at room temperature.

Procedure

The rabbits were randomly divided into two groups. One group was treated by intra-arterial administration of Lipiodol only (TAE-L group) and the other group was treated by administration of sorafenib in Lipiodol emulsion (TAE-S group).

Transarterial embolization was performed under fluoroscopy guidance at 2 weeks after implantation of VX2 carcinoma in the liver. After anesthesia was induced as described before, right auricular artery was punctured percutaneously and 4-F introducer (Micropuncture Access Set, Cook Medical, Bloominton, IN, USA) was inserted (Figure 1). Using a 2.0-F microcatheter (Progreat alpha,

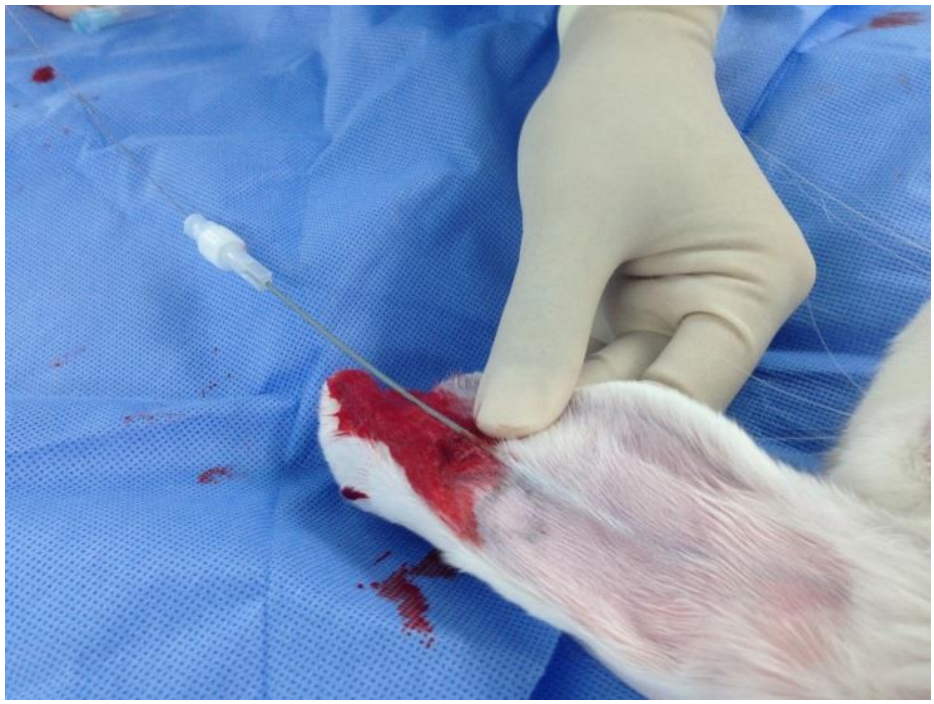
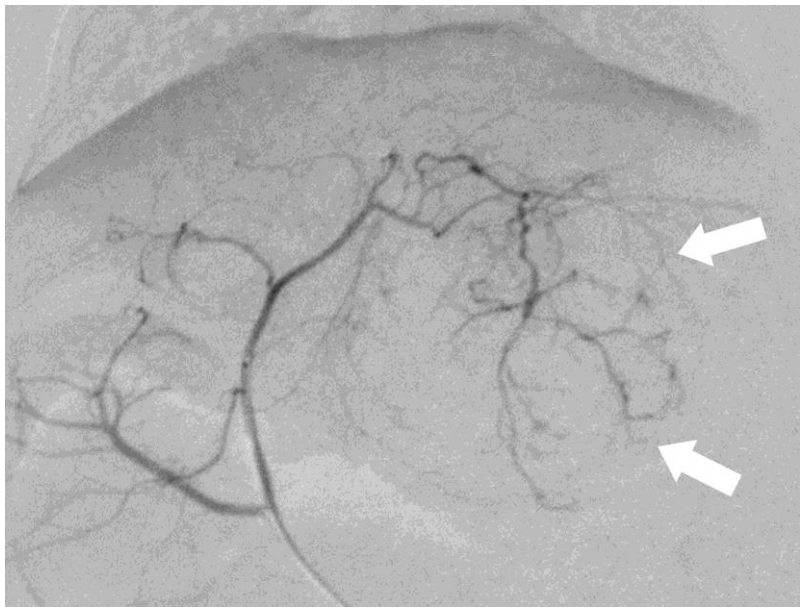
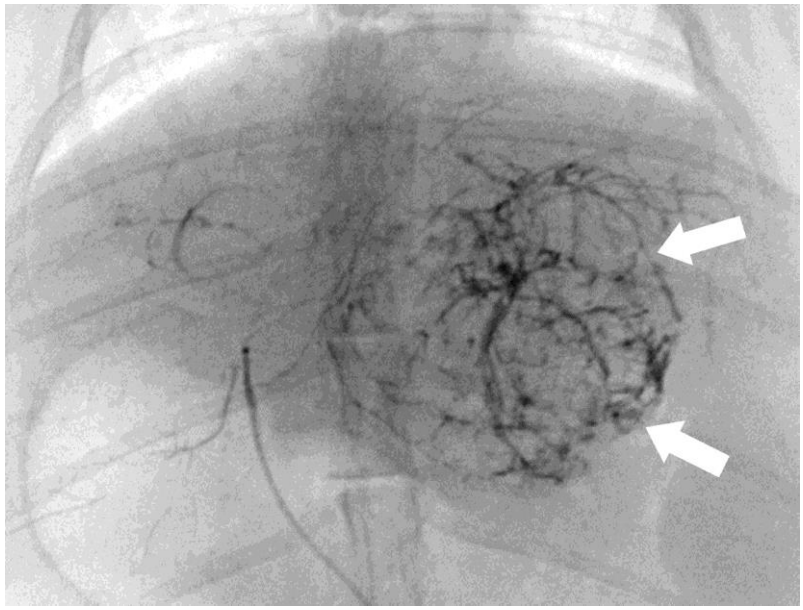


Figure 1. Access of right auricular artery using 4-F micro-introducer system.



(a)



(b)

Figure 2 Fluoroscopic findings. (a) Hepatic arteriography showed nodular tumor staining (arrows) in right lobe of the liver. (b) After chemoembolization, Lipiodol retention in the tumor (arrows) is noted.

Terumo, Tokyo, Japan) and guidewire (Meister, Asahi Intecc, Aichi, Japan), celiac angiography was performed for identification of hepatic arterial anatomy and the tumor (Figure 2). After selection of the left hepatic artery, 0.5 mL of Lipiodol (TAE-L group) or 0.5 mL of Sorafenib in Lipiodol emulsion (TAE-S group) were infused respectively. The catheter was then removed and hemostasis was made by manual compression.

Sampling Protocol

Peripheral blood was drawn from the left auricular artery before and at 0.5, 1, 2, 4, 24, and 72 hours after treatment in TAE-S group and before and at 24 and 72 hours after treatment in TAE-S group to measure serum sorafenib concentration and determine the hepatic toxicity caused by treatment. At 72 hours after treatment, necropsy was performed. A part of liver containing the tumor and non-tumorous parenchyma was sliced at a 5-mm interval and harvested livers were sliced to a thickness of 5mm and representative slices were fixed in 10% of neutralized formalin solution over 24 hours. A tumor cube with size of 5x5x5mm was obtained, weighed and homogenized using tissue homogenizer to measure tissue sorafenib concentration. Sampling protocol was summarized in Figure 3.

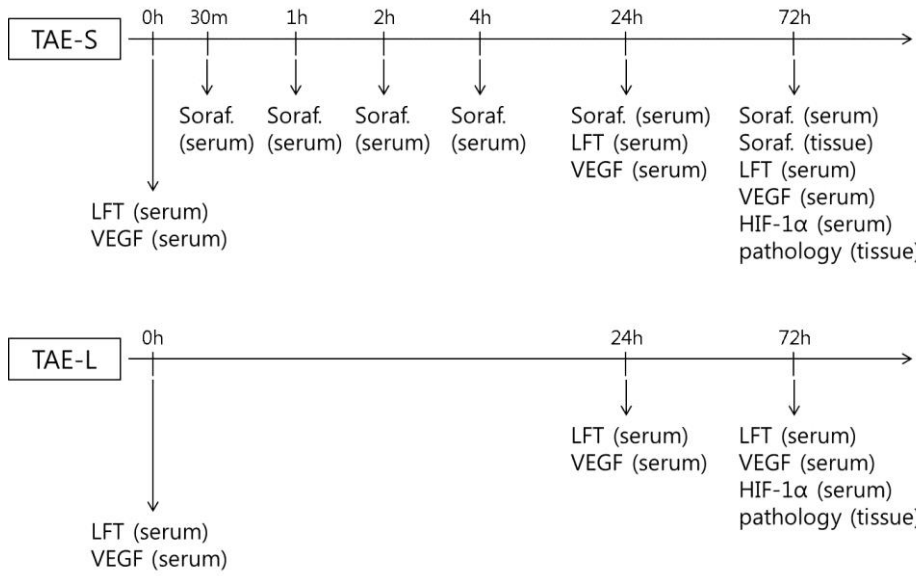


Figure 3. Sampling protocol

Measurement of Serum and Tissue Sorafenib Concentration

Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis was used to measure sorafenib concentrations, as previously described [4]. A Waters Alliance 2795 LC system and a MICROMASS Quattro micro API MS/MS spectrometer were used to carry out LC-MS/MS. The analysis parameters are as follows; for sorafenib, transition of mass-to-charge ratio (m/z) 464.9 to m/z 251.9; for sorafenib-D3 (as internal standard material), m/z 467.8 to m/z 254.95; desolvation temperature, 400℃; source temperature, 120℃; capillary voltage, 4.2 kV; cone voltage, 45 V; collision energy, 34 eV; nebulizing gas, nitrogen; collision gas, argon; column, XB-C_{18} column (2.6 ㎛, 4.6x75 mm); mobile phase, 0.01 mol/L ammonium acetate/acetonitrile mixture (35:65); flow rate, 0.5 mL/min. Sorafenib concentrations were measured in the serum at 0, 0.5, 1, 2, 4, 24, and 72 hours after treatment and in the tumor tissue harvested after necropsy at 72 hours after treatment.

Biochemical Assays

To evaluate the effect of each treatment on the hepatic function, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin levels were measured at different time points (0, 24, and

72 h). Serum VEGF and HIF-1α were also measured at the same time points (0, 24, and 72 h) by enzyme-linked immunosorbent assay (ELISA).

Histopathology

At 72 hours after treatment, rabbit necropsy was performed and livers were harvested. Gross diameter of the tumor was measured and representative specimens were fixed in 10% of neutralized formalin solution over 24 hours. After fixation, the samples were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) for histopathologic analysis. A pathologist who was blinded to the procedure performed, examined the slides under a light microscope. The following parameters were evaluated in the peritumoral hepatic parenchyma as previously described [10]; capsular inflammation, steatosis, portal inflammation, spotty necrosis, ballooning degeneration, sinusoid dilatation, and hemorrhage. A score from 0 (normal) to 3 (severe damage) was assigned to each parameter. The grade of coagulation necrosis of remaining liver parenchyma was also evaluated.

To quantitate the anti-tumor effects, digital images of each sections were obtained and the number of pixels included in area of viable tumor cell and necrosis were counted using a graphic software (Adobe Photoshop CS6, Adobe systems, San Jose, USA), and the fraction of tumor necrosis was determined.

Statistical Analysis

Statistical analysis was performed using a commercially available statistics program (SPSS statistics version 20.0; SPSS, USA). To evaluate temporal change of levels of hepatic enzymes and concentrations of angiogenetic factors within each group, Wilcoxon signed rank test was used. To compare tumor size, tumor necrosis, levels of hepatic enzymes, concentration of angiogenetic factors between the two groups, Mann-Whitney U test was used. Fisher's exact test was performed to compare the histopathologic parameters.

Results

Access of left hepatic artery was possible in all the rabbits and Lipiodol or emulsion of sorafenib and Lipiodol were delivered successfully as planned. There was no procedure-related mortality. Outcomes were summarized in Table 1.

Pharmacokinetics of Sorafenib

The mean sorafenib concentrations in the serum in TAE-S group were 62.7 ng/mL, 96.3 ng/mL, 121.0 ng/mL, 110.3 ng/mL, 80.2 ng/mL, and 30.8 ng/mL at 0.5, 1, 2, 4, 24, and 72 hours after treatment. Serum concentration peaked at 2 hours after treatment, and then gradually decreased. At 72 hours after treatment, mean concentration of serum sorafenib decreased to 30.8 ng/mL (Figure 4). Mean concentration of sorafenib in the tissue harvested at 72 hours was 10,217.3 ng/g (range, 482.4-10217.3), and mean tissue-to-serum ratio was 406.8 (range, 9.8-1400.8).

Biochemical Assay

Baseline serum AST, ALT, ALP, and total bilirubin levels did not show significant difference between TAE-L group and TAE-S group. Serum AST ($p=0.008$ in TAE-L group and $p=0.012$ in TAE-S group) and ALT ($p=0.008$ in

			TAE-L	TAE-S	p value
Body weight (kg)			3.14	3.14	0.971
Tumor size (cm)			2.1	2.3	0.436
Hepatic enzymes	ASL (IU/L)	0 h	19.7	22.1	0.968
		24h	243.6	715.4	0.036
		72h	207.3	161.1	0.113
	ALT (IU/L)	0 h	53.0	44.7	0.278
		24h	286.9	854.3	0.015
		72h	294.9	584.3	0.113
	ALP (IU/L)	0 h	148.5	123.2	0.093
		24h	195.2	355.8	0.059
		72h	193.3	220.1	0.720
	Bilirubin (mg/dL)	0 h	0.3	0.5	0.661
		24h	0.5	0.9	0.606
		72h	0.2	0.3	0.999
Angiogenetic factors	VEGF (pg/mL)	0 h	118.7	129.3	0.673
		24h	79.7	79.5	0.815
		72h	99.2	109.7	0.541
	HIF-1α (pg/mL)	0 h	103.4	137.0	0.252
		24h	102.2	142.0	0.036
		72h	144.7	142.8	0.743
Tumor necrosis (%)			56.6	83.9	0.011

Table 1. Mean values of body weight, biochemistry, tumor size, and degree of tumor necrosis

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *VEGF* vascular endothelial growth factor, *HIF-1α* hypoxia-inducible factor 1α

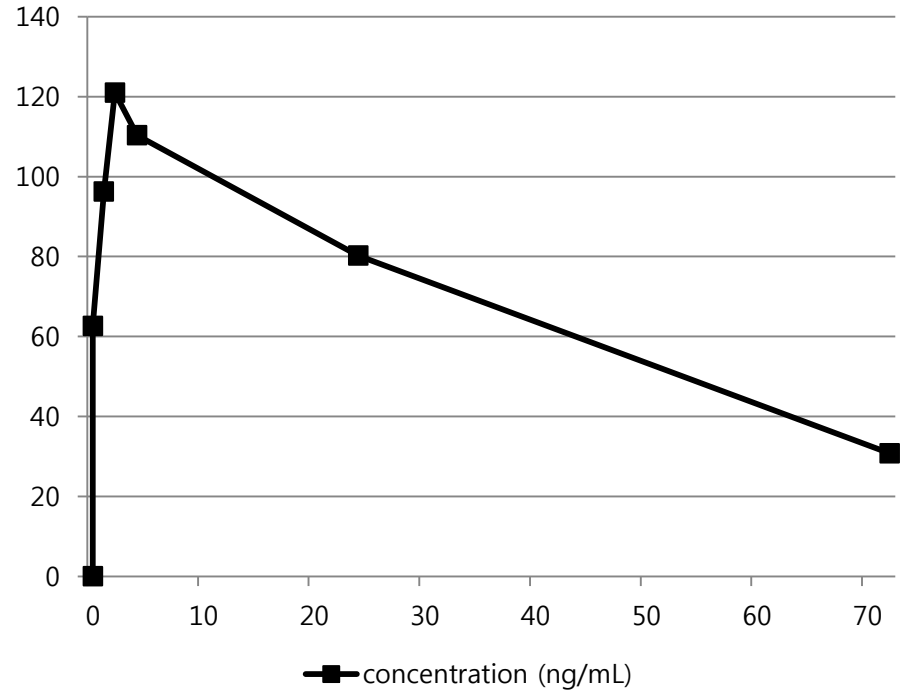
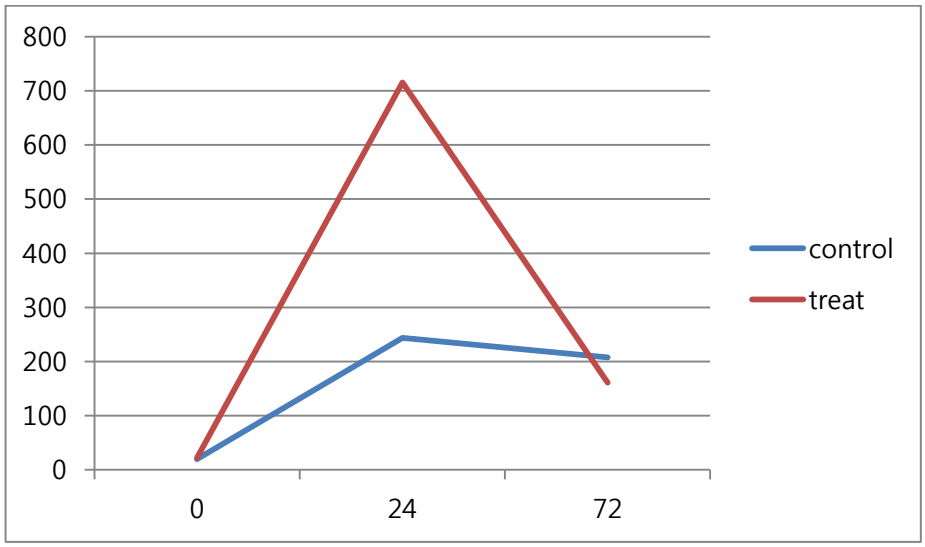


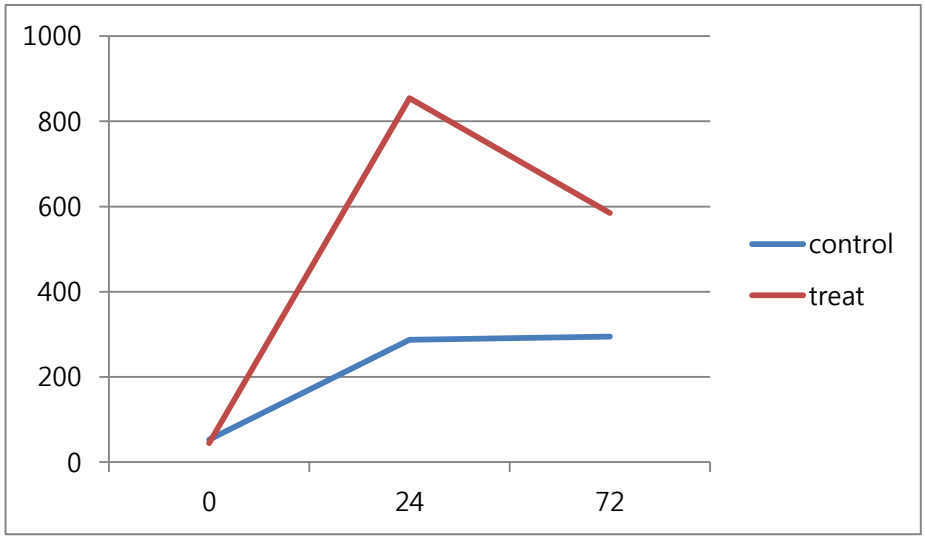
Figure 4. Concentration of serum sorafenib

TAE-L group and p=0.012 in TAE-S group) levels increased at 24 hours after treatment in both groups. AST and ALT of TAE-S group were significantly higher than those of TAE-L group (p=0.036 and p=0.0125, respectively) at 24 hours after treatment. The markers were decreasing at 72 hours after treatment, and there was no significant difference between two groups (Figure 5).

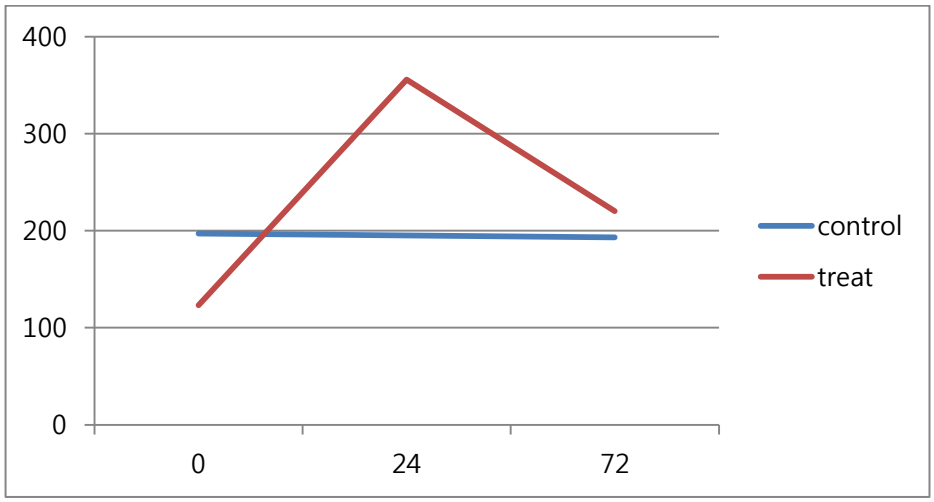
Mean serum VEGF concentration decreased at 24 hours after treatment in both groups (p=0.008 and p=0.012, respectively). Mean serum HIF-1α concentration at 24 hours after treatment, mean serum VEGF and HIF-1α concentrations at 72 hours after treatment showed no significant difference compared with their baseline values in each group. Mean serum VEGF and HIF-1α concentrations showed no significant difference between two groups at each time points (0, 24, and 72 hours), except HIF-1α concentration at 24 hours after treatment. HIF-1α concentration was higher in TAE-S group (142.0 pg/mL) than in TAE-L group (102.2 pg/mL; p=0.036) (Figure 6).


800
700
600
500
400
300
200
100
0
0
24
72
control
treat

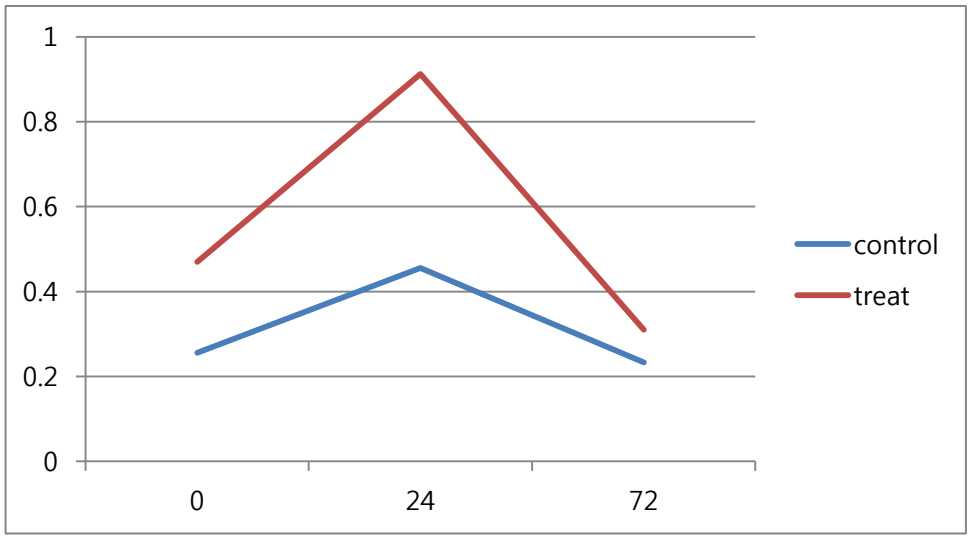
(a)


1000
800
600
400
200
0
0
24
72
control
treat

(b)

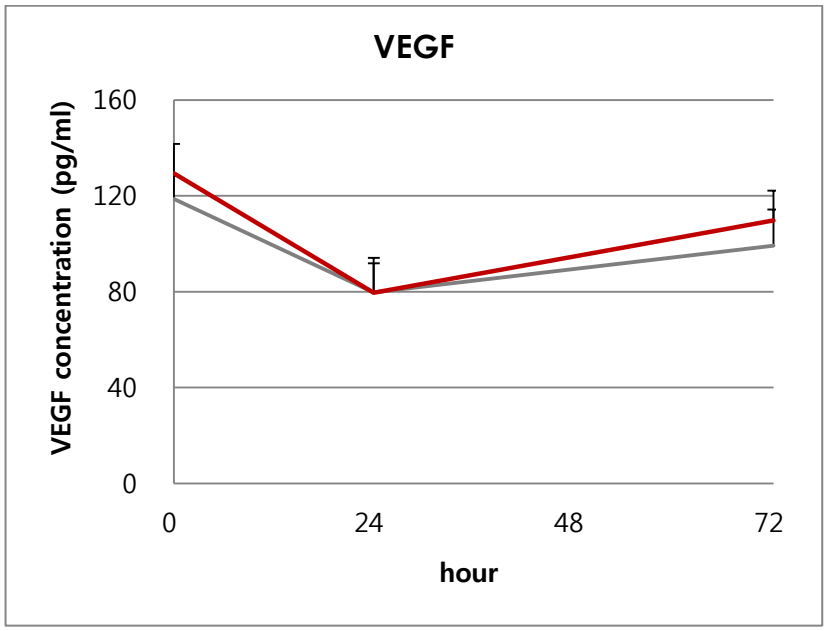

400
300
200
100
0
0
24
72
control
treat

(c)

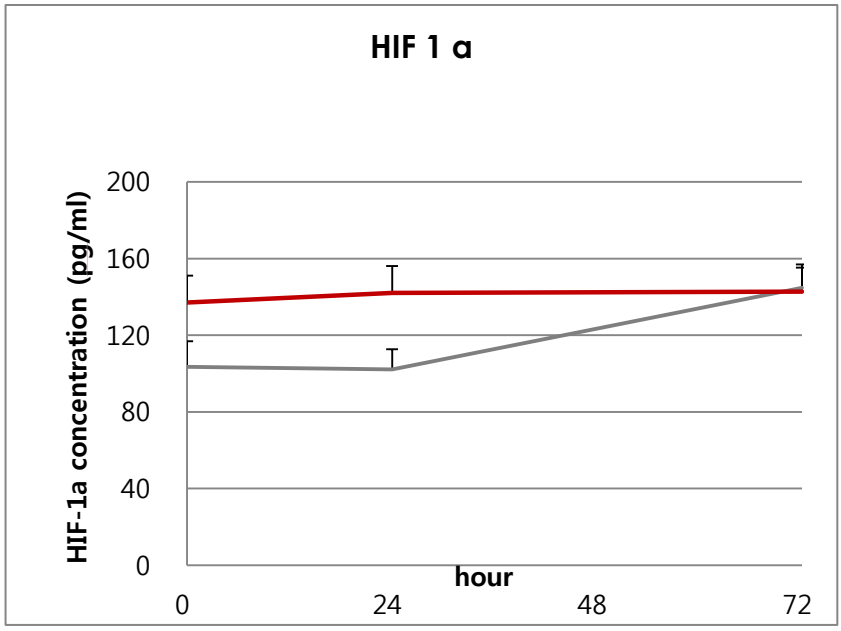

1
0.8
0.6
0.4
0.2
0
0
24
72
control
treat

(d)

Figure 5. Liver function test. Serum aspartate aminotransferase (AST) (a) and alanine aminotransferase (ALT) (b) levels increased at 24 hours after treatment in both groups. AST and ALT of TAE-S group were significantly higher than those of TAE-L group at 24 hours after treatment. Serum alkaline phosphatase (c) and bilirubin levels showed no significant difference (increase) along time and between groups.


VEGF
VEGF concentration (pg/ml)
160
120
80
40
0
0
24
48
72
hour

(a)


HIF 1 a
HIF-1a concentration (pg/ml)
200
160
120
80
40
0
hour
0
24
48
72

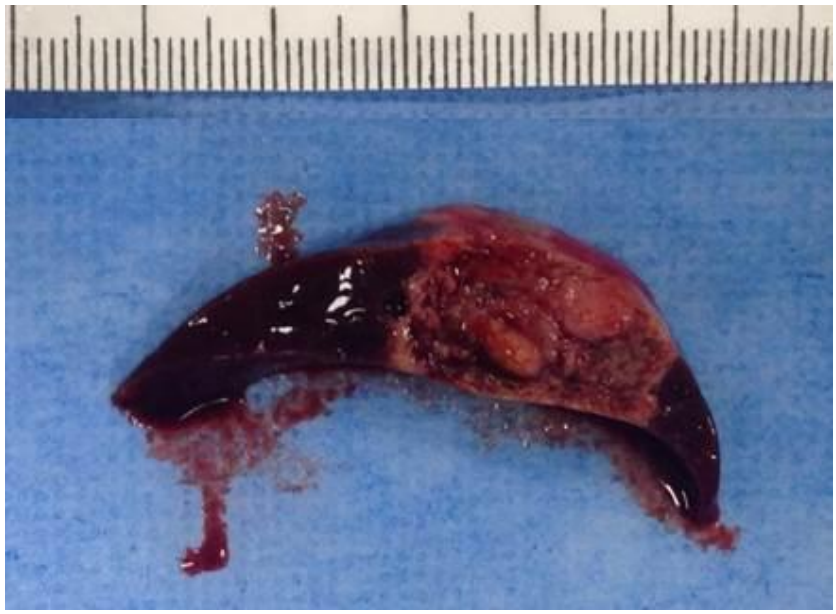
(b)

Figure 6. Mean serum concentration of VEGF (a) and HIF-1α (b). Red lines indicate TAE-S group and gray lines indicate TAE-L group. There was no statistically significant difference between groups.

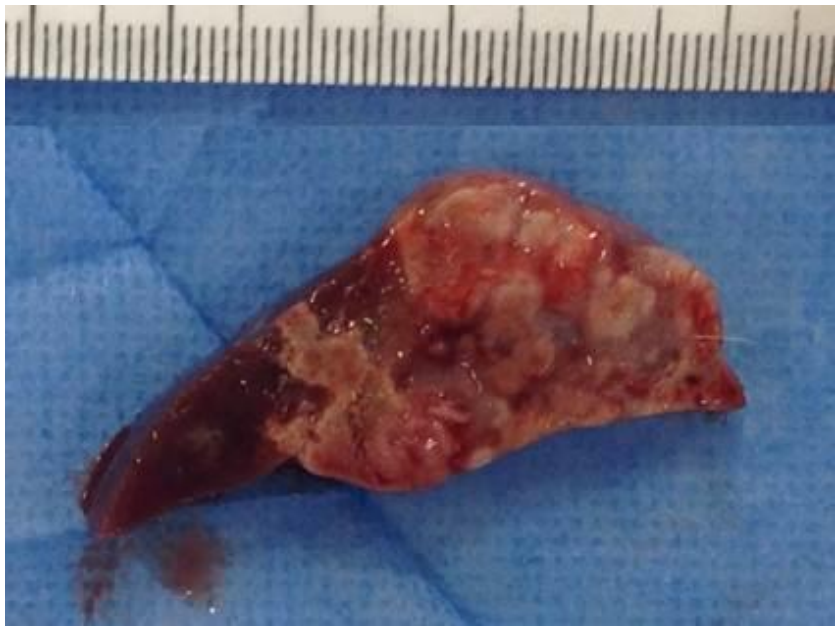
Histopathology

Degree of capsular inflammation, steatosis, portal inflammation, spotty necrosis, sinusoidal dilatation, and hemorrhage showed no significant difference between two groups (p=1.000, 1.000, 1.000, 0.303, 0.666, and 1.000, respectively). But degree of ballooning degeneration and coagulation necrosis of remaining liver parenchyma were more severe in TACE-s group (p=0.008 and 0.003, respectively).

The mean gross tumor size was 2.1cm in TACE-l group and 2.3cm in TACE-s group, and the difference was not statistically significant (p=0.436) (Table 2, Figure 7). Mean fraction of tumor necrosis after treatment was significantly higher in TAE-S group (83.9%) than in TAE-L group (56.6%) (p=0.011) (Table 2, Figure 8)



(a)

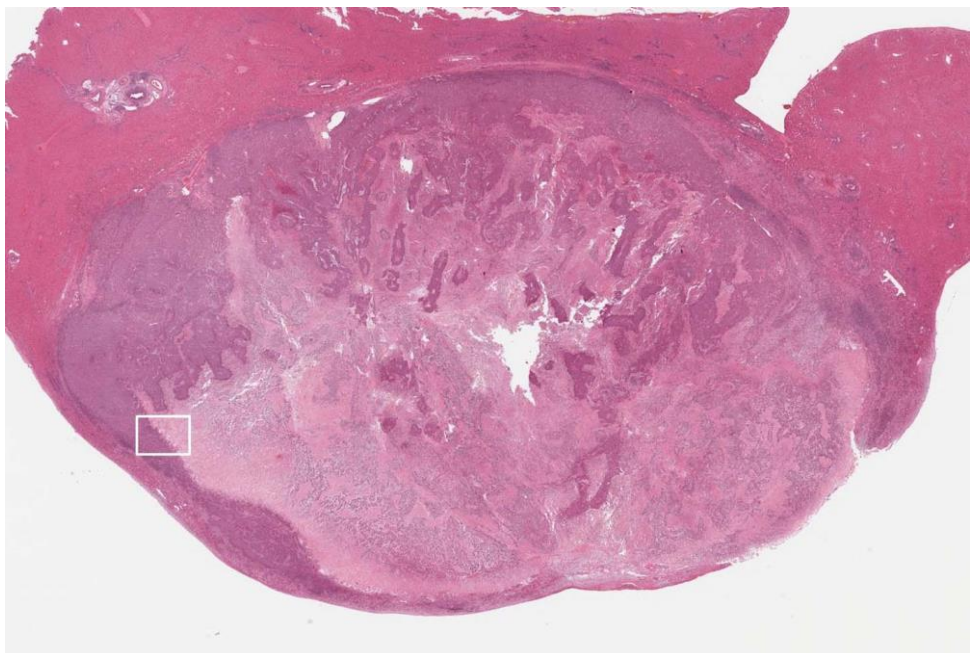


(b)

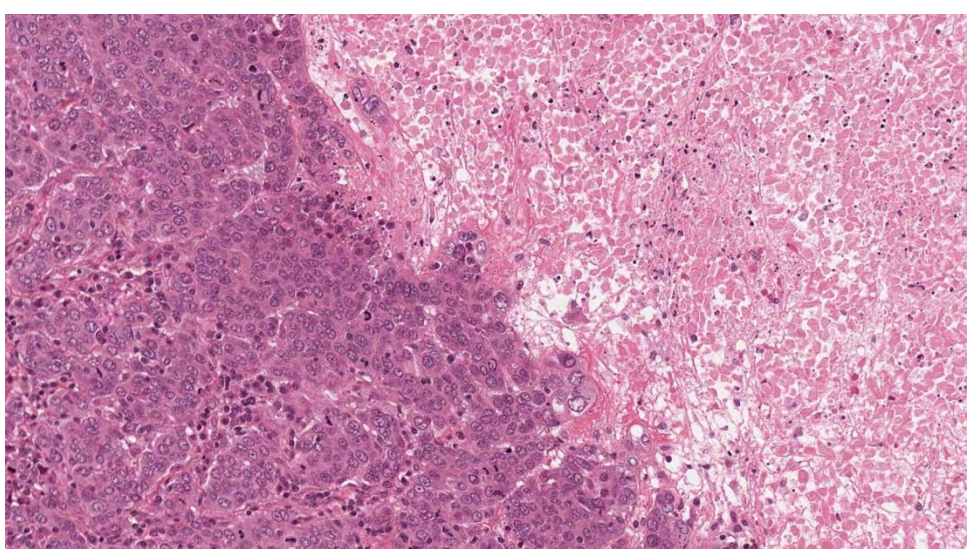
Figure 7. Representative gross pathologic findings of TAE-L group (a) and TAE-S group (b).

Rabbit Number	TAE-L		TAE-S	
	Size (cm)	Necrosis (%)	Size (cm)	Necrosis (%)
1	2.2	42	3.3	96
2	1.7	44	2.6	63
3	1.3	16	2.1	79
4	2.7	48	2.3	95
5	2	67	2.2	81
6	1.9	91	1.4	92
7	3.1	49	2.9	84
8	2.6	51	2.3	83
9	1.4	93	1.6	77
10	2.1	65	2.6	89
Mean	2.1	56.6	2.3	83.9

Table 2. Tumor size and fraction of necrosis. The difference of gross tumor size was not statistically significant (p=0.436). Mean fraction of tumor necrosis after treatment was significantly higher in TAE-S group (83.9%) than in TAE-L group (56.6%) (p=0.011).



(a)



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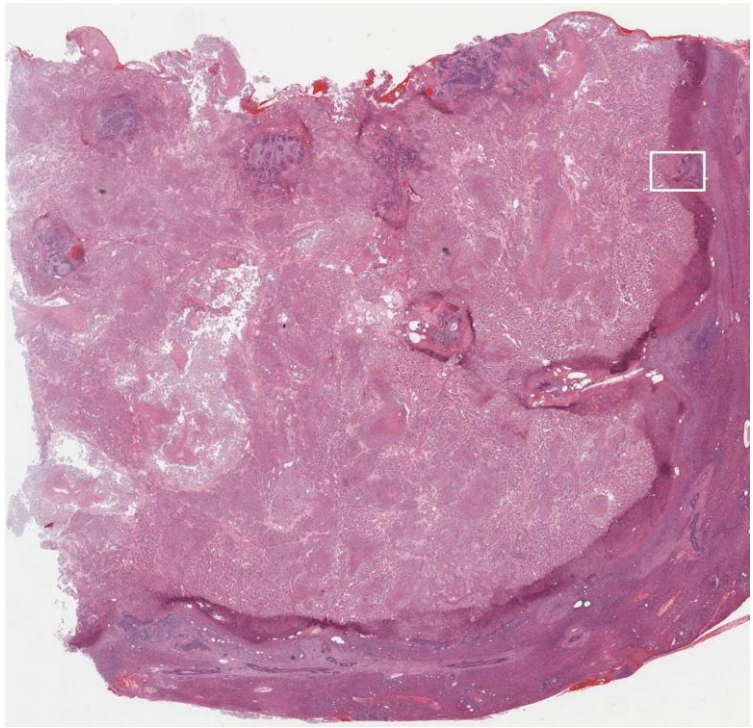
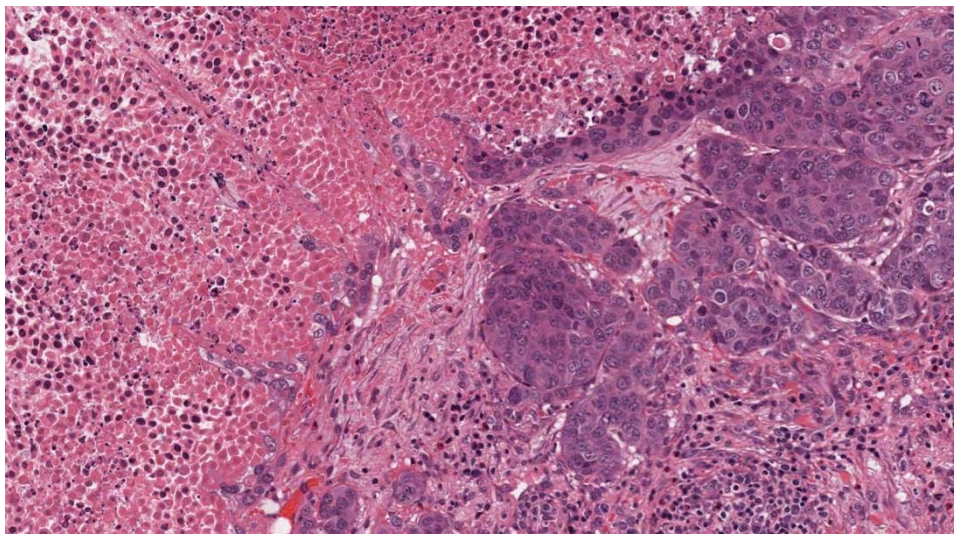


(c)



(d)

Figure 8. H&E stains of representative specimens. (a) A low-power field image from TAE-L group. Necrosis occupies 67% of the tumor area. (b) High-power field image of annotated area in (a) shows the border between viable tumor and necrotic portion. (c) A low-power field image from TAE-S group. Necrosis occupies 95% of the tumor area. (d) High-power field image of annotated area in (c) shows the border between viable tumor and necrotic portion.

Discussion

Pharmacokinetics of sorafenib in our study showed similar result to previously published data [1; 4; 12], which supported the strength of transarterial delivery of sorafenib compared to systemic delivery in the view of enhancing localized effect. Sorafenib concentration in the tissue was much higher than that in the serum till 72hours after treatment. We measured more frequently in the early times after treatment to enhance temporal resolution of pharmacokinetics, and revealed that the concentration of sorafenib in the serum peaked at 2 hours after treatment.

Contrary to previous studies which reported that sorafenib TACE was safe and there were no or minimal deterioration of hepatic function [1; 12; 19], AST and ALT levels were significantly higher in TACE-s group at 24 hours after treatment in our study. Possible explanation would be the difference in dose and preparation method of sorafenib. In terms of dose, we delivered 10mg of sorafenib per rabbit, which is much higher dose than that in the study of Chatziioannou et al. [1], and similar dose (i.e. 3mg/kg) to other studies [4; 12; 19]. For the preparation of sorafenib, we sonicated the mixture of sorafenib powder and Lipiodol according to Chatziioannou's method, on the other hand, sorafenib was prepared as solution dissolved in a solvent mixture described in the other studies. So, we hypothesized that the concentration of sorafenib in Lipiodol was too high to make clear solution with Chatziionnou's method [1], and sorafenib delivered as a form of suspension containing powders might play a role as an embolic material, resulted in liver

damage.

At 72 hours after treatment, all the hepatic enzyme levels of TAE-S group decreased and showed no significant difference with those of TAE-L group. But the degree of ballooning degeneration and coagulation necrosis of the peritumoral liver were severe in TAE-S group, indicating hepatic parenchyma of TAE-S group got more damage after TACE. Because the patients with HCC have poor hepatic function due to underlying liver cirrhosis and are susceptible to the hepatic damage, careful consideration is needed for preparation and dose of sorafenib for intraarterial administration. Though superselective TACE is almost impossible in the small rabbits, we should put effort into perform TACE as selective as possible to reduce hepatic damage in clinical application.

Several *ex vivo* studies showed that sorafenib inhibited HIF-1α protein accumulation in the HCC cells and decreased VEGF expression [6; 17]. In the clinical study, the SHARP trial had reported that none of the biomarkers tested, including VEGF, significantly predicted response to sorafenib [7]. Another study reported that only 22.2% of patients who received sorafenib treatment had a VEGF decrease [13]. We investigated the possibility that VEGF and HIF-1α could be a biomarker predicting effect of sorafenib TACE, but the results showed that serum VEGF and HIF-1α levels after treatment were not different whether sorafenib was administered or not. Thus, VEGF or HIF-1α cannot be a surrogate biomarker of the effect of sorafenib.

Previous studies on sorafenib TACE had focused only on pharmacokinetics and safety in the normal liver [1; 4] and in a VX2 tumor model [12]. To our best knowledge, Zhang's publication is the only study on the effect of sorafenib TACE in rabbit VX2 tumor model [19]. They assessed microvessel density and showed decrease of angiogenesis after sorafenib TACE, and also revealed the treatment attenuated tumor growth and intrahepatic metastasis. We focused on the necrosis of the tumor itself as an effect of sorafenib TACE, and found that sorafenib TACE induced tumor necrosis more than Lipiodol TACE.

We measured the sorafenib concentrations in short intervals immediately after embolization to enhance temporal resolution of pharmacokinetics and revealed that the concentration of sorafenib in the serum peaked at 2 hours after treatment. We focused on the pharmacokinetics of sorafenib within 72 hours after TACE. We think the effect of sorafenib is more important in the early period after TACE. TACE induces immediate hypoxia to the HCC cells and can upregulate angiogenesis. So Recent clinical studies on the combination therapy of TACE and oral administration of sorafenib concern the possibility of "rebound" resulted from 3-4 days of sorafenib interruption before and after TACE [11] and investigate combined TACE with uninterrupted sorafenib therapy [3].

Though we observed more tumor necrosis in TAE-S group, VEGF and HIF-1α did not differ significantly in our study and we did not perform other angiogenesis study, such as microvessel density. Thus we cannot say that intraarterial

administration of sorafenib ‘actually’ inhibited tumor angiogenesis, and the possibility that embolic effect of sorafenib emulsion may affect tumor necrosis cannot be excluded.

Conclusions

Transarterial administration of sorafenib was feasible in VX2 model of rabbits, resulting in high tumoral concentration of sorafenib. Degree of tumor necrosis was significantly higher in TAE-S group than in TAE-L group, but more severe toxicity of normal liver tissue was observed.

References

1. Chatziioannou AN, Siskos AP, Loxas D, et al. (2013) Transarterial embolization with sorafenib in animal livers: a pharmacokinetics study. J Vasc Interv Radiol, 24(11):1657-1663 e1651
2. Cheng AL, Kang YK, Chen Z, et al. (2009) Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol, 10(1):25-34
3. Cosgrove DP, Reyes DK, Pawlik TM, Feng AL, Kamel IR, Geschwind JF (2015) Open-Label Single-Arm Phase II Trial of Sorafenib Therapy with Drug-eluting Bead Transarterial Chemoembolization in Patients with Unresectable Hepatocellular Carcinoma: Clinical Results. Radiology, 277(2):594-603
4. Gaba RC, Yap FY, Martinez EM, et al. (2013) Transarterial sorafenib chemoembolization: preliminary study of technical feasibility in a rabbit model. J Vasc Interv Radiol, 24(5):744-750
5. Liu L, Chen H, Wang M, et al. (2014) Combination therapy of sorafenib and TACE for unresectable HCC: a systematic review and meta-analysis. PloS one, 9(3):e91124
6. Liu LP, Ho RL, Chen GG, Lai PB (2012) Sorafenib inhibits hypoxia-inducible factor-1alpha synthesis: implications for antiangiogenic activity

in hepatocellular carcinoma. Clin Cancer Res, 18(20):5662-5671

7. Llovet JM, Pena CE, Lathia CD, et al. (2012) Plasma biomarkers as predictors of outcome in patients with advanced hepatocellular carcinoma. Clin Cancer Res, 18(8):2290-2300

8. Llovet JM, Ricci S, Mazzaferro V, et al. (2008) Sorafenib in advanced hepatocellular carcinoma. N Engl J Med, 359(4):378-390

9. Lo CM, Ngan H, Tso WK, et al. (2002) Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. Hepatology, 35(5):1164-1171

10. Muftuoglu MA, Aktekin A, Ozdemir NC, Saglam A (2006) Liver injury in sepsis and abdominal compartment syndrome in rats. Surg Today, 36(6):519-524

11. Park JW, Koh YH, Kim HB, et al. (2012) Phase II study of concurrent transarterial chemoembolization and sorafenib in patients with unresectable hepatocellular carcinoma. J Hepatol, 56(6):1336-1342

12. Parvinian A, Casadaban LC, Hauck ZZ, van Breemen RB, Gaba RC (2015) Pharmacokinetic study of conventional sorafenib chemoembolization in a rabbit VX2 liver tumor model. Diagn Interv Radiol (Ank), 21(3):235-240

13. Tsuchiya K, Asahina Y, Matsuda S, et al. (2014) Changes in plasma vascular endothelial growth factor at 8 weeks after sorafenib administration as predictors of survival for advanced hepatocellular carcinoma. Cancer, 120(2):229-237

14. Wang B, Xu H, Gao ZQ, Ning HF, Sun YQ, Cao GW (2008) Increased expression of vascular endothelial growth factor in hepatocellular carcinoma after transcatheter arterial chemoembolization. Acta Radiol, 49(5):523-529

15. Wilhelm SM, Carter C, Tang L, et al. (2004) BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res, 64(19):7099-7109

16. Wu XZ, Xie GR, Chen D (2007) Hypoxia and hepatocellular carcinoma: The therapeutic target for hepatocellular carcinoma. J Gastroenterol Hepatol, 22(8):1178-1182

17. Xu M, Zheng YL, Xie XY, et al. (2014) Sorafenib blocks the HIF-1alpha/VEGFA pathway, inhibits tumor invasion, and induces apoptosis in hepatoma cells. DNA Cell Biol, 33(5):275-281

18. Xu W, Kwon JH, Moon YH, et al. (2014) Influence of preoperative transcatheter arterial chemoembolization on gene expression in the HIF-1alpha pathway in patients with hepatocellular carcinoma. J Cancer Res Clin Oncol, 140(9):1507-1515

19. Zhang L, Liu FY, Fu JX, Duan F, Fan QS, Wang MQ (2014) Hepatic arterial administration of sorafenib and iodized oil effectively attenuates tumor growth and intrahepatic metastasis in rabbit VX2 hepatocellular carcinoma model. Int J Clin Exp Pathol, 7(11):7775-7781

요약(국문초록)

목적 : 본 연구의 목적은 토끼 VX2 간 종양 모델에서 소라페닙을 이용한 간동맥화학색전술의 가능성, 안전성 및 항종양 효과를 평가하는 것이다.

재료 및 방법 : 간 좌엽에 VX2 종양을 이식한 총 20마리의 New Zealand white rabbit을 2군으로 나누어 실험하였다. 한 군은 간동맥을 통하여 0.5mL의 요오드화 오일 (리피오돌)만을 투여하였으며(TAE-L 군), 다른 한 군은 0.5mL의 리피오돌과 10mg의 소라페닙 이멀젼을 투여하였다(TAE-S 군). TAE-S군에서 liquid chromatography tandem mass spectrometry를 이용하여 말초혈 혈청에서의 소라페닙 농도를 실험 전과 실험 0.5, 1, 2, 4, 24 및 72시간 후에 측정하였으며, 또한 실험 72시간 후에 획득한 종양 조직 내에서의 소라페닙 농도도 측정하였다. 혈청내 간효소치, 혈관내피성장인자 (vascular endothelial growth factor, VEGF), 저산소-유발 인자 1α (hypoxia-inducible factor 1α, HIF-1α)를 실험 전과 실험 24시간 및 72시간 후에 측정하였다. 조직병리학적 검사를 시행하여 종양의 괴사 정도 및 정상 간조직 손상 정도를 평가하였다.

결과 : 혈청 내 소라페닙 농도는 실험 후 2시간째에 최고에

도달하였다. 72시간째에 측정한 조직내 소라페닙 농도는 같은 시간대 혈청내 소라페닙 농도에 비해 평균 406.8배 높았다. AST와 ALT 농도는 기저치에 비해 두 군 모두에서 실험 후 24시간째에 유의하게 증가하였으며, TAE-S 군에서 TAE-L 군에 비해 유의하게 높았다. 혈청내 VEGF와 HIF-1α 농도는 두 군 간에 유의한 차이가 없었다. 풍선화 변성 (ballooning degeneration) 및 잔존 간 조직의 응고 괴사 (coagulation necrosis) 정도는 TAE-S 군에서 더 심하였다. 실험 후 종양의 괴사 정도는 TAE-S 군(83.9%)에서 TAE-L 군(56.6%)보다 유의하게 높았다(p=0.011).

결론 : 토끼 VX2 간 종양 모델에서 소라페닙을 이용한 간동맥화학색전술은 실현 가능하였으며, 간 내에서 전신 순환에 비해 높은 소라페닙 농도를 유도할 수 있었다. 종양의 괴사 정도도 TAE-S 군에서 유의하게 높았다. 그러나 정상 간 조직에 보다 심한 독성이 나타났다.

중심단어 : 소라페닙, 간동맥화학색전술, 간암, VX2 종양

학번 : 2010-30497