Preliminary Report

The Effect of Doxorubicin on the Changes of Serum Vascular Endothelial Growth Factor (VEGF) in Patients with Hepatocellular Carcinoma after Transcatheter Arterial Chemoembolization (TACE)

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Background: *Treatment of hepatocellular carcinoma (HCC) with transcatheter arterial chemoembolization (TACE) is known to induce vascular endothelial growth factor (VEGF) expression. A recent study has shown that doxorubicin can repress hypoxic induction of VEGF expression in human cancer cells.*
Objective: *To evaluate the combination effects of doxorubicin and TACE on the change of serum VEGF after TACE.*
Material and Method: *Thirty patients with unresectable HCC were assigned into two groups, the experiment group (n = 15) received TACE with doxorubicin (25-50 mg) plus mitomycin C (5-10 mg), and the control group (n = 15) received TACE with mitomycin C (5-10 mg). Serum VEGF before and after TACE (24 hour) was measured by quantitative sandwich enzyme-linked immunosorbent assay.*
Results: *Baseline serum VEGF was correlated with the size of tumor ($r^2 = 0.85$; $p = 0.03$). In addition, serum VEGF was significantly elevated after TACE ($p = 0.014$). However, the change of serum VEGF after TACE is not statistically different in both groups ($p = 0.72$). At 2-years, the overall survival was 38% and 40% in the experiment and control group, respectively ($p = 0.48$).*
Conclusion: *The present study suggests that doxorubicin improves neither the level of serum VEGF nor the survival in HCC patients treated with TACE.*

Keywords: *Doxorubicin, hepatocellular carcinoma, mitomycin C, transcatheter arterial chemoembolization, vascular endothelial growth factor*

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A recent study suggested that TACE might contribute to angiogenesis of HCC, possibly due to anoxic stress and ischemia-reperfusion injury[1,2]. Vascular endothelial growth factor (VEGF) is a major factor contributes in the process of angiogenesis[3,4]. A previous study demonstrated that doxorubicin could repress hypoxic induction of VEGF expression in human cancer cells[5]. Consequently, the authors examined the activity of doxorubicin in suppression of VEGF expression, as a combination therapy in HCC patients treated with TACE.

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Material and Method

The protocol was approved by the institutional review board (Rajavithi Hospital and National Cancer Center, Thailand). The present study population

consisted of patients with unresectable HCC. The inclusion criteria were as follows: (1) no obstruction of the main portal trunk; (2) Child's class A or B; (3) Karnofsky performance status > 90%; and (4) no prior TACE or chemotherapy.

TACE

All patients were assigned to one of two treatment groups (experiment and control groups) (Fig. 1). In both groups, TACE was performed with infusion of a mixture of ionized oil contrast medium, and Ivalon particles. In the experiment group, patients received doxorubicin (25-50 mg) plus mitomycin C (5-10 mg) as chemotherapeutic agents for TACE and in the control group, patients received mitomycin C (5-10 mg).

Detection of circulating VEGF

Venous blood was drawn from HCC patients 24 h before and after TACE. Tubes were centrifuged at 3000 g for 10 min. Serum was separated and stored at -80°C until VEGF assay by enzyme-linked immunosorbent assay (ELISA) (R&D System, Minneapolis, USA). Serum VEGF per platelet count were used to correct variation of serum VEGF levels in patients with different platelet counts.

Statistical method

The change of serum VEGF before and after TACE was determined using the paired Student's *t*-test. The survival analysis was calculated according to the method of Kaplan-Meier[6]. The log rank was used in the analyses of survival outcome. The relationships between circulating VEGF levels and other variables, *t*-test, Chi-square test, Fisher exact test and correlation coefficient (*r*) were used when appropriate. $p < 0.05$ was considered statistically significant.

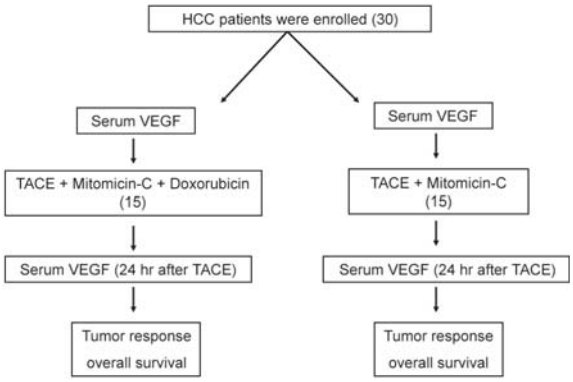

Fig. 1 Study schema. Patients with unresectable HCC were enrolled onto one of two arms of treatment. Serum VEGF was measured at 24 hour before and after TACE

Results

Correlation between serum VEGF and clinical features in the patients with HCC

Between January and December 2007, thirty patients were eligible for data-analysis, 15 patients were selected to the experiment group and the others were in the control group. The clinical characteristics of the patients are demonstrated in Table 1. No statistically significant differences in patient characteristics were observed.

Serum VEGF level was increased with the size of the tumor ($r^2 = 0.85$; $p = 0.03$) (Fig. 2). However, there was no correlation between serum VEGF and the clinical features, including age, serum albumin, bilirubin, AFP level and clinical child's classification.

Change of serum VEGF level after TACE

Serum VEGF level was significantly elevated in patients with HCC after TACE (0.97 ± 0.16) compared

Table 1. Demographic data of study patients prior to TACE

	Control group	Doxorubicin group	p-value
Sex (male:female)	12:3	13:2	0.40
Age (yr)*	52 (40-65)	59 (37-65)	0.22
Tumor size (cm)**	6.90 ± 4.30	4.20 ± 30.00	0.72
Serum AFP (IU/dL)**	21,894.60 ± 902.30	12,664.16 ± 952.60	0.64
Base line serum VEGF (pg/mL)**	150.00 ± 122.00	100.00 ± 111.00	0.24
Base line platelet (cell/mL)**	212.80 ± 94.70	196.00 ± 131.80	0.74
Base line serum VEGF/platelet**	0.75 ± 0.50	0.67 ± 0.62	0.50

* Median (range)
** Mean ± SD

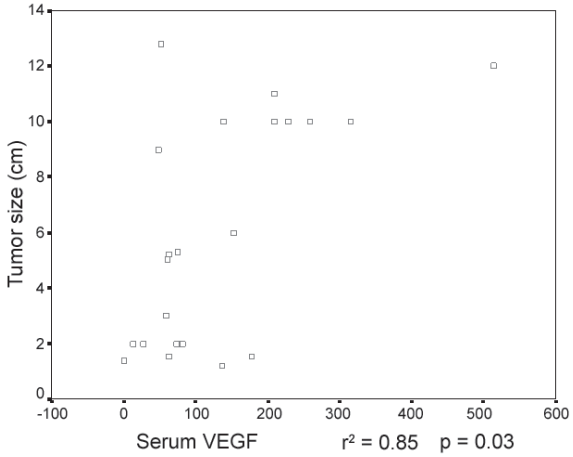

Fig. 2 Correlation between serum VEGF concentration and tumor size. A significantly positive correlation between serum VEGF concentration and tumor size, is shown ($r^2 = 0.85$; $p = 0.03$)

to pre-TACE (0.70 ± 0.50) ($p = 0.014$). The authors observed that the serum VEGF level was significantly elevated in both groups (control group: 0.75 ± 0.55 vs. 0.97 ± 0.14; $p = 0.015$ and doxorubicin group: 0.67 ± 0.62 vs. 0.94 ± 0.22; $p = 0.026$) (Fig. 3). However, the difference of the serum VEGF levels was not significant between the two treatment groups ($p = 0.722$).

Treatment response was evaluated according to the change in tumor volume more than 50% and retention of the oil. There was no difference between the responding of treatments in both arms of the treatment (Fig. 4).

Overall survival and survival rates

The median survival of all patients calculated from the date of TACE treatment was 16 months. The Kaplan-Meier survival algorithm yielded a survival probability of 38% in the experiment group and of 40% in the control group after 24 months. However, the differences of survival probabilities were not statistically significant in both groups ($p = 0.48$) (Fig. 5).

Discussion

A previous study demonstrated that VEGF levels were significantly elevated in patients with HCC on the first post-TACE day then VEGF level decreased gradually on the third day[7]. Hence, the authors measured the VEGF level on 24 hours after TACE. The presented data confirmed the evidence that VEGF was produced and released in HCC patients treated with

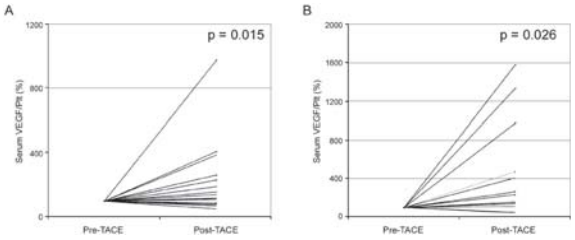

Fig. 3 Percent change in serum VEGF after TACE. Value of 100% represents baseline serum VEGF 24 hour before TACE. (A) and (B) Percent change where patients were treated either in experimental groups or in control group

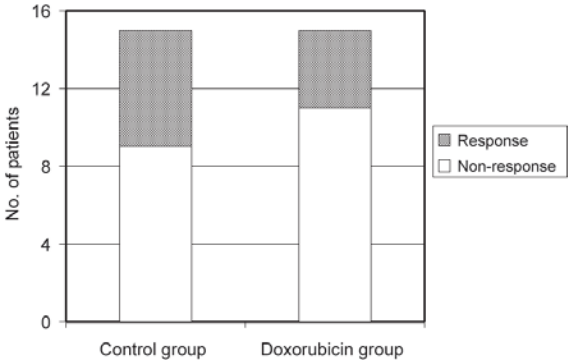

Fig. 4 Treatment response was evaluated at 6 week after TACE (according to the change in tumor volume more than 50% and retention of the oil)

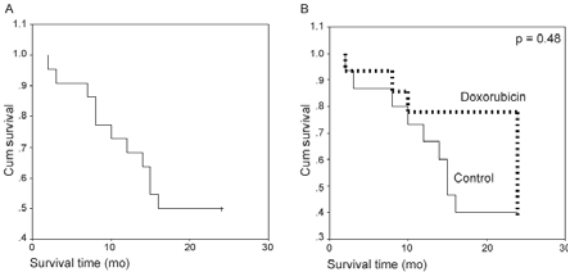

Fig. 5 Overall survival (OS) estimates using the Kaplan-Meier method. OS rate at 24 months was 38% among patients assigned to the experimental (doxorubicin plus TACE) group and 40% among patients treated with TACE, control group, ($p = 0.48$)

TACE[8, 9]. However, the change of serum VEGF before and after TACE was not different between the two groups of treatment. What the present results actually

suggested is that doxorubicin failed to suppress the expression of VEGF after TACE. Addition of doxorubicin does not improve the overall survival rate of HCC patients treated with TACE. However, the present study has some limitations. The number of patients enrolled was small. The present study suggested that further study with a larger patient population and using more efficient antiangiogenic drugs is warranted to clarify its value in clinical application.

In conclusion, the present study suggests that doxorubicin improves neither the level of serum VEGF nor the overall survival in HCC patients treated with TACE.

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ผลของ doxorubicin ต่อการเปลี่ยนแปลงระดับ VEGF พื้นฐานในซีรัมผู้ป่วยโรคมะเร็งตับ HCC ภายหลังการทำ transcatheter arterial chemoembolization

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ภูมิหลัง: การรักษาโรคมะเร็งตับด้วย transcatheter arterial chemoembolization (TACE) ชักนำให้มีการเพิ่มขึ้นของซีรัม VEGF มีการศึกษาที่แสดงให้เห็นว่า doxorubicin สามารถยับยั้งการแสดงออกของ VEGF ในเซลล์มะเร็งบางชนิดได้

วัตถุประสงค์: เพื่อศึกษาผลของ doxorubicin และ TACE ต่อการเปลี่ยนแปลงระดับ VEGF พื้นฐาน VEGF ในซีรัมผู้ป่วยหลังรับการรักษาด้วยวิธี TACE

วัสดุและวิธีการ: ผู้ป่วยโรคมะเร็งตับชนิด HCC ในระยะที่ไม่สามารถรับการผ่าตัดรักษา จำนวน 30 ราย ถูกแบ่งออกเป็น 2 กลุ่ม คือกลุ่มทดลองผู้ป่วยได้รับการรักษาด้วย TACE ร่วมกับได้รับ doxorubicin (25-30 mg) และ mitomycin (5-10 mg) จำนวน 15 ราย และกลุ่มควบคุมผู้ป่วยได้รับการรักษาด้วย TACE ร่วมกับ mitomycin C (5-10 mg) จำนวน 15 ราย ผู้นิพนธ์ทำการเก็บซีรัมของผู้ป่วยก่อนและหลังการรักษาด้วย TACE (24 ชั่วโมง) เพื่อวัดระดับ VEGE ในซีรัมด้วยวิธี quantitative sandwich emzyme-linked immunosorbent assay

ผลการศึกษา: ระดับของ VEGF ในซีรัมมีความสัมพันธ์เชิงเส้นตรงกับขนาดของมะเร็งตับ ($r^2 = 0.85$; $p = 0.03$) และระดับของ VEGF ในซีรัมมีการเพิ่มขึ้นอย่างมีนัยสำคัญ ภายหลังจากการรักษาด้วย TACE ($p = 0.014$) แต่อย่างไรก็ตามระดับของ VEGF ในซีรัมภายหลังทำ TACE ทั้งกลุ่มทดลองและกลุ่มควบคุมไม่แตกต่างกันทางสถิติ ($p = 0.72$) โดยผู้ป่วยมีอัตราการรอดชีวิตที่ 2 ปีเท่ากับ 38% และ 40% ในกลุ่มทดลอง และกลุ่มควบคุมตามลำดับ ($p = 0.48$)

สรุป: จากการศึกษานี้แสดงให้เห็นว่า doxorubicin ไม่มีผลต่อการเปลี่ยนแปลงของระดับ VEGF ในซีรัม และอัตราการรอดชีวิตที่ 2 ปีในผู้ป่วยโรคมะเร็งตับซึ่งได้รับการรักษาด้วยวิธี TACE